The behavioural need for lying and feeding in the high yielding dairy cow

Thesis

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The behavioural need for lying and feeding in the high yielding dairy cow

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Abstract

Experiments were conducted to examine the impact of high milk yields on the behaviour and welfare of dairy cows. A preliminary study determined the social interactions existing within a group of 60 cows from which sub-samples of 20 high and 20 low yielding cows, that were not observed to interact with each other, were identified. These cows were used to examine the behavioural strategies employed by dairy cows coping with increased nutritional demands from milk production. Grazing time significantly increased with milk yield, consequently reducing lying time. A second study measured the appetite of high and low yielding cows for concentrates, using operant conditioning. The high yielding cows demonstrated no greater appetite for concentrates, but post-testing behavioural observations indicated greater appetite for forage, suggesting increased motivation to feed. Subsequent experiments investigated the behavioural effects of lying deprivation on dairy cattle welfare, further examining the feeding-lying conflict. Cows forced to stand had reduced milk yields and demonstrated behavioural signs of fatigue, frustration and stress, which were cumulative. When deprived of lying, a post-deprivation increase in this activity was achieved by reducing feeding time. When deprived of both lying and feeding, a feeding, not lying, post-deprivation increase was observed. However, lying was not reduced to extend feeding time. In conclusion, the motivation to feed was greater in high yielding dairy cows. This resulted in extended grazing and a reduced lying time. Lying can be regarded as an important and highly motivated behaviour and may compromise welfare if reduced.

The legitimacy of using individual dairy cows as replicates in the statistical analysis of their behaviour was also investigated. There was no significant difference in the coefficients of variation between cows that were free to interact, and those that were not, for a range of behaviours. Therefore, those cows able to interact did not appear to influence each other's behaviour, supporting the use of individual dairy cows as replicates.

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1. Introduction

The purpose of this research programme was to examine the impact of high milk yields on the behaviour and welfare of dairy cows. The possibility of behavioural interdependence existing between dairy cows was also integral to this study and has been considered in both experimental design and statistical analysis.

Today's dairy cow is far removed from the life and productive capacity of its feral counterpart. Webster (1987) suggests that technology, along with greater knowledge about animal production and the health benefits of milk, have all played a part in creating the modern dairy cow. The dairy cow of today eats more, metabolises food at a greater rate (FAWC, 1997), and as a consequence, is bigger, heavier and produces more milk.

This increase in milk yield is primarily due to better nutrition (increased feed quality and rationing) and the use of genetic selection (Harrison, *et al.*, 1990). Genetic technology is responsible for bringing about the largest increases in milk production (Webster, 1995). Although these changes have brought about benefits to the farmer, such as improved production efficiency and an increased income, the impact of these changes on the animal's welfare is unclear. Welfare organisations have expressed a concern regarding high yielding dairy cows and the FAWC (1997) commented that '[milk] production may have already passed the point where good cow welfare can be maintained'.

Evidence for this has been concluded both from an assessment of the animal's physical condition and observations of the time spent in food-directed behaviours.

Kamphues (1998), for example, stated that dairy cows, especially high yielders, are unable to consume sufficient energy to satisfy their appetite. This may be particularly the case for grazing dairy cows. The benefits of a high space allowance and a clean, open environment may be offset by the failure of grazing cows to consume sufficient herbage to sustain a high yield, with the result that the cow can become emaciated as she mobilises body fat reserves (Phillips, 2001).

Phillips (1998) noted that the most variable grazing period for dairy cows is at night. This is determined, in part, by the cow's milk yield whereby high yielders take a 'midnight snack'. It is unusual for cows to graze at night due to a fear of predators (Phillips, 1998) or difficulties in herbage selection (Phillips & Hecheimi, 1989). This suggests that high yielding cows may have a high motivation to graze at this time, which is likely to stem from an increased appetite and the feeling of hunger (Phillips, 1993). Phillips and Denne (1988) also observed that extensively managed high yielders have to extend their grazing period to consume sufficient amounts of herbage to support their level of production. This is probably achieved by grazing earlier in the morning (Phillips & Rind, 2002) and later at night, in comparison to their lower yielding counterparts (Phillips & Denne, 1988). In addition, the high yielder may increase feed intake by increasing biting rate and dry matter intake per bite (Phillips, 2001).

This evidence suggests that the grazing high yielder may suffer from the unpleasant emotional state of persistent hunger, which is due to an inability to consume sufficient herbage to sustain a high level of milk production. The high yielder may therefore have to employ a range of coping strategies in order to satisfy the demands of milk

production. However, the adoption of these strategies may result in the cow having to neglect the expression of other important behaviours such as lying down to rest (Phillips, 1993). As cattle indicate a strong motivation to lie down (Metz, 1985) a reduction in this behaviour is likely to compromise their welfare and may also demonstrate the severity of hunger being experienced.

It was therefore the aim of this project to examine the extent to which a high milk yield affects the behaviour and welfare of the grazing dairy cow.

2. Literature review

2.1 Animal welfare issues

2.1.1 Animal needs and requirements

All animals have needs, which aid the survival and continuation of the species. A 'need' is a necessity, or a necessary requirement that has to be satisfied in order to maintain good welfare. There are two different kinds of needs as discussed by Dawkins (1983a), these are:

(i) *Ultimate:* If the animal went without these, it would die. For example, food or water.

(ii) *Proximate:* If the animal went without these, it would suffer - but not necessarily die. For example, the opportunity to socialize and graze in the dairy cow.

The needs of animals have been recognised as essential components of welfare and have therefore formed the basis of the Five Freedoms (FAWC, 1997) (Table 2.1).

Table 2.1 The five freedoms (FAWC, 1997)

1. Freedom from Thirst, Hunger and Malnutrition
2. Freedom from Thermal and Physical Discomfort
3. Freedom from Pain, Injury and Disease
4. Freedom to Express Normal Behaviour
5. Freedom from Fear and Distress

Ensuring the needs of the animal will not only improve welfare but, in general, conform to high economic output and animal performance (Von Borell, 1998).

Science can find out what an animal's needs are and devise ways of satisfying them. Behavioural psychology is a branch of science that enables us to understand the animal's mind and find out what matters to it most. A number of ways to assess and measure needs in animals have been determined. Such methods include preference studies and motivation tests, which will be discussed in more detail later in this report. Methods of assessing needs include:

i. Examining what an animal does when it has an element of free choice in its environment (Broom & Johnson, 1993), *i.e.* preference tests.

ii. Examining innate behaviours and those behaviours that are required to satisfy physiological needs (Phillips, 1993).

iii. Observing which behaviour an animal performs after being deprived of performing two or more major behaviours (Phillips, 1993).

iv. Recording the amount of time an animal is engaged in a particular activity when observed in its 'natural' environment, *i.e.* time budgets.

Using the framework outlined in the second method above, Phillips (1993) concluded that the major behavioural needs of cattle can be categorised as follows: Reaction to

danger (flight & escape), ingestion, body care (including elimination), motion, exploration/territorialism, rest, and association (including socialisation and coitus).

Measuring motivation is very useful as it can be used to identify the resources or stimuli in the environment that matter most, and are therefore important, to an animal. It is possible to determine how hard an animal is willing to work for something, and therefore attach a relative value to it.

If animals are unable to satisfy their needs they may suffer physically and/or psychologically (Dawkins, 1990). The prevention of a major behaviour may result in frustration and the performance of abnormal behaviours (Dawkins, 1990). Animals may therefore employ coping strategies in order to deal with inadequacies in their environment. The severity of the inadequacy will determine the degree and severity of the coping strategy employed. If the environment is, for example, severely restricting the performance of a major behaviour over a period of time, this coping strategy may be interpreted as an abnormal or aberrant behaviour. Broom and Johnson (1993) defined this as a 'behaviour which differs in pattern, frequency or context from that which is shown by most members of the species in conditions which allow a full range of behaviour.' This would be an obvious display of poor welfare. However, coping strategies may not be so severe. For example, if cattle are left to graze on a pasture with a low sward, then they may simply graze for longer and bite faster in attempting to meet their feed intake (Phillips, 1993). Trying to determine welfare here is more difficult, but could be considered not so severe.

There are various behavioural problems that can be employed by the animal as coping strategies. These are categorised as follows:

- *Stereotypies*: Fox (1965) defined these as 'unvarying, repetitive behaviour patterns that have no obvious goal or function.' These can be seen in animals that are housed in confined environments with little enrichment. Here, the animal's ability to perform certain behaviour patterns is restricted. Therefore, the animal is motivated to perform a particular behaviour but is unable to and as a result becomes frustrated (Rushen *et al.*, 1993). Examples include tongue rolling in cattle and weaving in horses.

- *Injurious behaviour*: These are behaviours that cause injuries to the animal itself or to others. This is common in environmentally deficient, group housing systems. Examples include tail biting in pigs and excessive mounting in cattle.

- *Redirected behaviour*: Fraser & Broom (1997) describe this as 'the direction of some behaviour, such as an act of aggression, away from the primary target and toward another, less appropriate object.' For example, an insufficient diet will produce cravings for a nutrient that is lacking. If this nutrient is fibre, for example, then the animal may redirect the motivation to obtain fibre by chewing wood (Phillips, 1993).

Thus, it is important that we understand animals by determining their needs and therefore improve their welfare by catering for them.

The aim of this review is to determine the behavioural needs of the high yielding dairy cow and explore and suggest possible measures to assess these. As an example, the high yielder may be deprived of social contact or rest due to the extra time spent grazing in order to consume sufficient herbage to sustain body condition.

2.1.2 Defining welfare

Animal welfare is a complex subject and multi-disciplinary in its approach. Some confusion comes from being unable to satisfactorily define the term 'welfare'. To help understand the concept, similar words that contribute to the theme of welfare can be listed. Such words may include well-being, health (mental, physical and physiological), feelings/emotions, balance - *i.e.* biological - between the animal and its surroundings, and harmony - with the environment.

A useful definition has been given by Broom (1986) who defines the welfare of an animal as '...its *state* as regards its attempts to *cope* with its *environment*'. The word 'state' is concerned with the psychological, physiological and physical components of the animal. The definition indicates that each of these factors can be reduced or enhanced depending on the situation the animal is in. An influence on any one of these factors will alter the well-being of that animal. The term 'cope' indicates a struggle to live. If this struggle is too severe then welfare will be reduced (Broom, 1986). However, the reverse is also true, as suffering can arise due to the inability of an animal to make a difference to the way it is living, for example, by making changes to its environment. This is what Webster (1995) termed 'hopelessness.' The 'environment' component is everything with which the animal interacts. This may be another animal, stockmen or aspects of the housing environment. English and

McPherson (1994) have devised a useful diagram to cover those environmental components that impact on the animal and influence its welfare (Fig. 2.1). As can be seen from Fig. 2.1, animal welfare is not based on one factor but is a collection and combination of many. These elements are essential to the welfare of livestock.

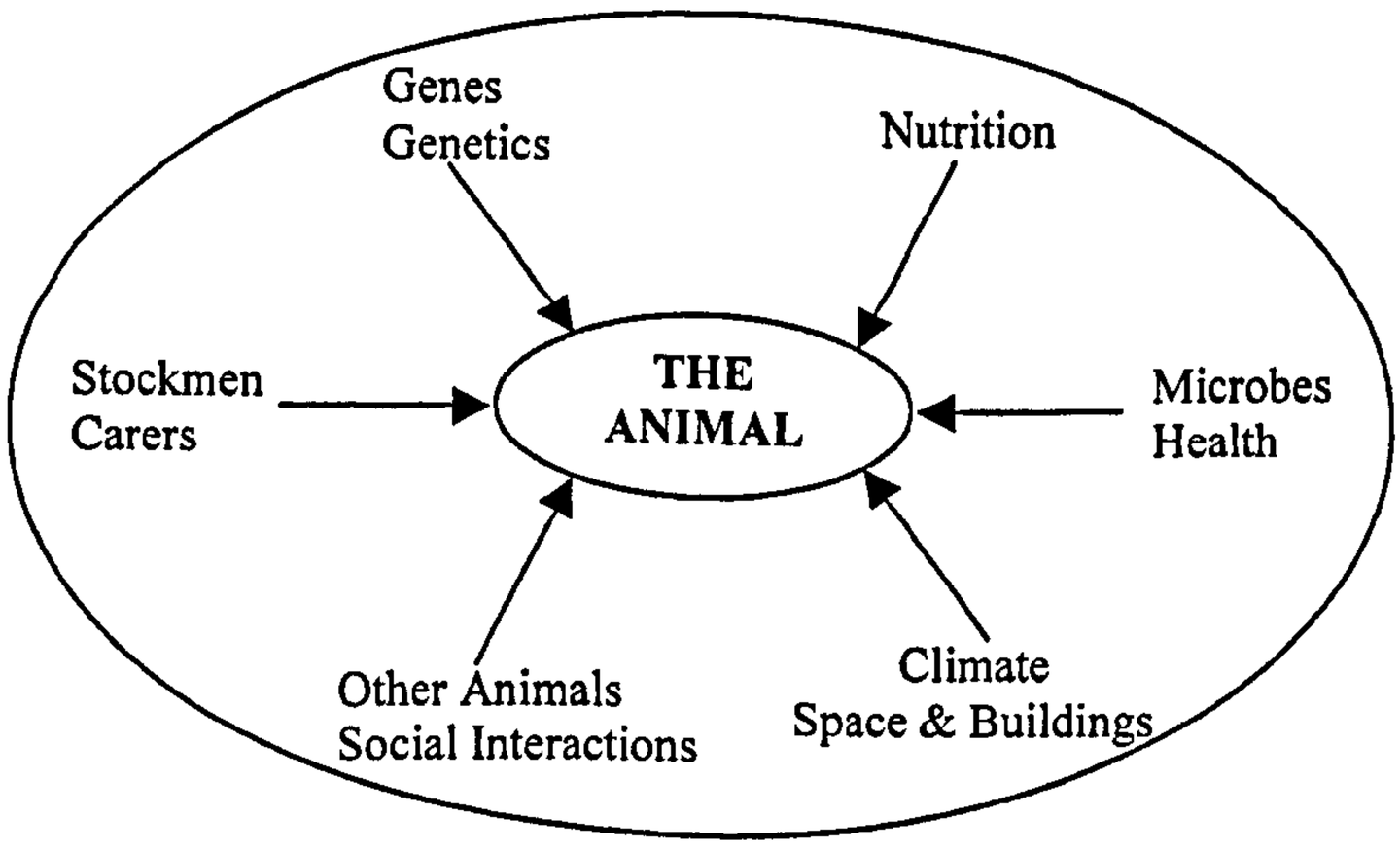


Fig. 2.1 The major controlling influences impacting on an animal's welfare (English & McPherson, 1994)

It is a generally held view that welfare is concerned with, and centered around, the term 'suffering' (Rushen & de Passille, 1992), which is defined as 'an unpleasant, aversive *mental* or *emotional* state.' This, then, assumes that welfare is very much involved with the mental states of animals and therefore involves a psychological perspective. Dawkins (1990) also explained that welfare is only of concern if an animal is experiencing unpleasant mental experiences.

The previous section (2.1.1) discussed the proposition that needs are of two kinds, *i.e.* ultimate and proximate. Duncan (1990), on the other hand, used the terms 'needs' and

'wants', respectively. Duncan (1990) stated that welfare is primarily concerned with 'wants' as opposed to 'needs'. This is also in agreement with Webster's (1990) definition of welfare as being determined by our perception of 'the animal's perception of its environment'. This would suggest that welfare is independent of satisfying 'needs' (as defined by those factors that ensure the survival and continuation of the species). Moreover, welfare is concerned with what the animal thinks [it needs] and is therefore dependant on satisfying 'wants'.

Alternative views suggest that welfare is concerned with the biological functioning and health of animals. For example, Broom (1991) indicated that it is biological fitness (*e.g.* reproductive success) that underpins good welfare. Similar definitions include the themes of good health, physiological needs and normal development as major constituents of animal welfare.

The Five Freedoms (Table 2.1) cater for both views *i.e.* physiological and psychological and provide a useful framework in considering the welfare of an animal. In addition, English and Edwards (1992) have described welfare as being categorised into physical and psychological aspects (Table 2.2) but have noted that they, to some extent, interact with each other. Some definitions also identify with both arguments. For example, Hughes (1976) defined welfare as 'a state of complete mental and physiological health where the animal is in harmony with its environment.'

It would seem that the psychological state of an animal *is* welfare, whereas the 'alternative views' (mentioned above) are factors that might *impinge on* welfare.

Therefore, welfare could be considered as an individual's state of mind (relating to the emotional content of an animal *i.e.* its feelings). This is altered by the animal's ability to cope with its environment. The animal's ability to cope will depend on its previous experiences and the opportunities that exist within the environment to satisfy its behavioural and physiological needs. The degree by which an animal is able to cope will determine its welfare.

Table 2.2 Physical (a) and psychological (b) aspects of welfare as defined by English & Edwards (1992)

(a) Physical examples:
• Good biological performance • Good Health *e.g. absence of disease and parasites* • Adequate feeding *e.g. absence of nutritional deficiencies & maintenance of good body condition* • Good housing *i.e. physical & thermal comfort*

(b) Psychological examples:
• Absence of fear *i.e. from environment; other animals; or humans* • Ability to control or have a choice of environment • Cater for innate requirements of the animal *i.e. certain behaviour patterns and physical needs*

This suggests that welfare should be judged using the subjective experiences of animals, as it can be argued that if an animal does not perceive (mentally process) itself to be in a poor state of welfare, then there is no welfare problem concerned with that animal. From this rationale, access to the private experiences of animals is

required in order to make decisions on their level of welfare. This information can be obtained from studies such as preference testing, which get as close to the animal's subjective experiences as is currently possible (Dawkins, 1983a). It is possible to use such techniques to 'ask' animals subtle questions in order to understand the world from their perspective. This can help get closer to understanding an animal and determining its welfare state (see arrow 'P' (preference testing) Fig. 2.2) prior to any indicators of poor welfare being shown. These indicators are indirect interpretations of the subjective feelings of animals and include factors such as reproductive success, adrenal activity and abnormal behaviours (English & Edwards, 1992) (Fig. 2.2).

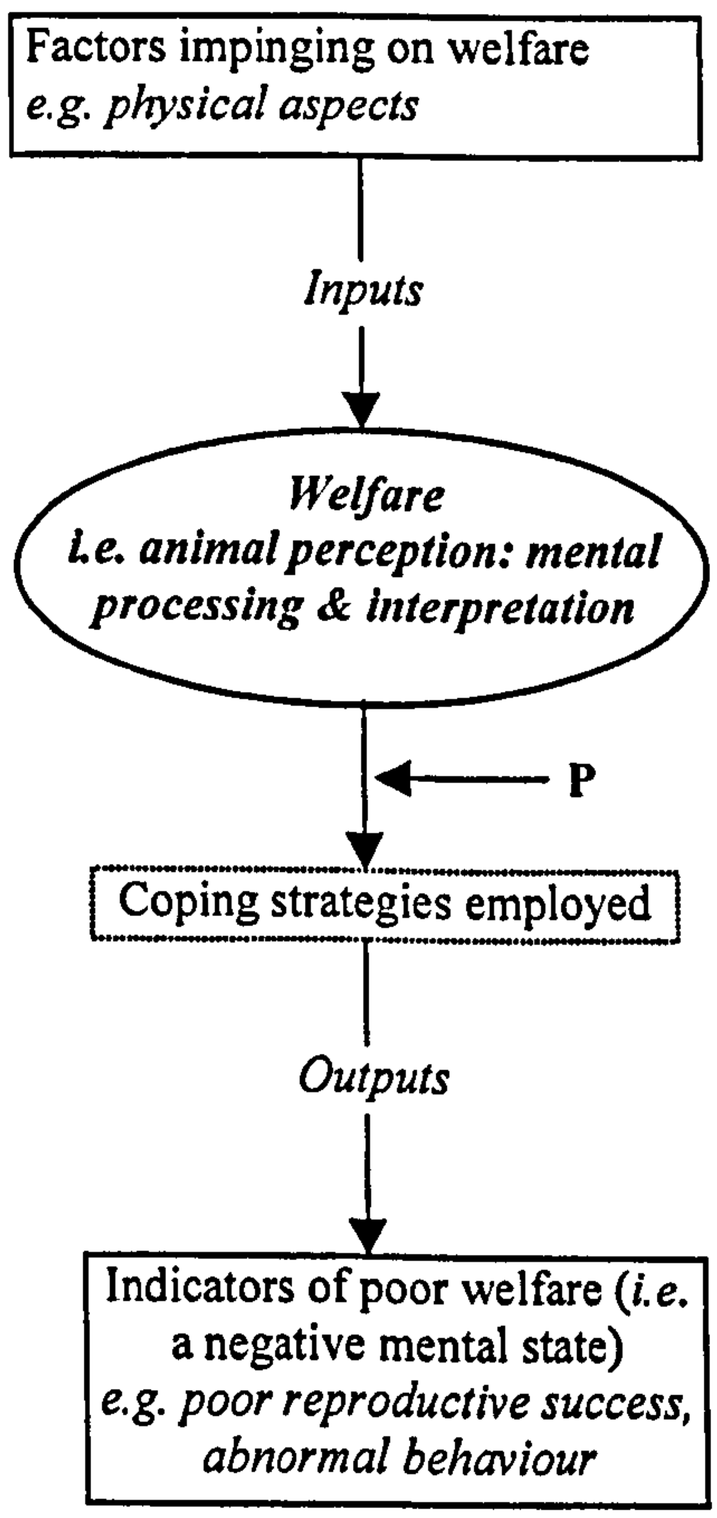


Fig. 2.2 Welfare is a state of mind and interpretation should therefore be based on the subjective experiences of animals (Dawkins, 1990; Rushen & de Passile, 1992).

As an example, the presence of a human will bring about an individual, mental experience within the animal, such as fear. It is important to mention here that welfare is concerned with the individual; each animal has been subject to its own unique learning experiences and will therefore interpret situations differently according to these (Broom, 1991). Coping mechanisms will be employed, such as avoiding contact and seeking refuge (behavioural) and increasing heart rate and respiration (physiological) to deal with this stressor. If these coping mechanisms are unable to satisfy the degree of stress encountered, or the environment does not provide the opportunity for stress relief, then a reduced level of welfare will be experienced.

Finally, the interpretation of animal welfare is constrained by the observer. As Ewbank (1999) points out 'value-judgements, to some extent, depend on the beliefs of the judgement-maker and they may change over time as the individuals beliefs change.' These beliefs will evolve as knowledge about animal welfare is increased. Decisions will be based on moral considerations formed from scientific investigations. In this light, as Phillips (1993) suggested, welfare can be 'considered as the human perception of the quality of life that an animal has, particularly in relation to its success in coping with its environment.'

2.2 The high yielding dairy cow

Today's dairy cow is far removed from the life and productive capacity of its feral counterpart. Comparing the productive parameters of the feral ruminant on an open range with that of a typical European Holstein/Friesian dairy cow reveals some stark differences (Table 2.3).

Table 2.3 Comparison of productive parameters between the feral and European dairy cow (Webster, 1993a)

	Feral	Domesticated
Milk production ($l\ d^{-1}$)	8 - 10	30 – 40
Number of milkings per day	4 - 6	2 – 3
Total lactation yield (litres)	< 1000	6,000 – 12,000
Max. amount of milk contained in udder at any one time (litres)	2	20+

Webster (1987) suggested that technology, along with knowledge about animal production and the health benefits of milk, have all played a part in creating today's dairy cow. The modern dairy cow eats more, metabolises food at a greater rate (FAWC, 1997) and, as a consequence, is bigger, heavier and produces more milk. This increase in milk yield is primarily due to better nutrition (increased quality and rationing) and the use of genetic selection (Harrison *et al.*, 1990). Genetic technology is responsible for bringing about the largest increases in milk production (Harrison *et al.*, 1990). From the 1970s to 1995 the average yield per cow per year increased from 3,750 to 5,395 litres and is still increasing (FAWC, 1997). Although these changes have brought about benefits to the farmer (*i.e.* improved production efficiency and an increased income) this has been at the expense of the cows' welfare. High yielders have a high metabolic turnover, which is associated with an increased risk of metabolic stress resulting in a variety of production diseases or nutritional imbalances (Webster, 1987). Selection for a high milk yield is therefore associated with an increased risk of health problems (Gearhart *et al.*, 1990) and thus poor welfare.

The welfare problems encountered by the high yielder are not new. Wilson (1978) noted similar problems (*i.e.* production diseases, lameness, and mastitis) in high

yielders in the 1970s when milk yields were considerably lower. Welfare organisations have expressed a concern about these problems and FAWC (1997) commented that 'production may have already passed the point where good cow welfare can be maintained.' Therefore, FAWC (1997) have suggested that consideration should be given to health traits (e.g. lameness, mastitis, and infertility) when breeding programmes are designed, *i.e.* selection for a higher milk yield should be a secondary consideration after selection for health and welfare traits.

2.2.1 Welfare problems of the high yielding dairy cow

Rauw *et al.* (1998) noted that animals selected for high production efficiency are 'more at risk from behavioural, physiological and immunological problems.' These all have consequences for welfare. Dairy cattle selection is primarily centered on producing a cow with a high milk yield. There are two main pathways by which the welfare of the cow can be affected as a result of this selection process:

1. Welfare aspects that are directly related to the selected trait, *e.g.* mastitis, lameness.

2. Those welfare issues that occur as an indirect effect of the selected trait. The selection for one trait, *e.g.* increased milk yield, may result in the selection of another [undesirable] trait that reduces welfare (*e.g.* infertility).

Selection for increased milk production brings little or no benefit to the cow and has a negative impact on her welfare overall (Phillips, 1997) (Table 2.4). The effects these increases in yield have on the welfare of the high yielder will be discussed in the subsequent sections.

Table 2.4 Effects of selecting for milk yield on various welfare parameters (Adapted from Phillips, 1997)

	Welfare Parameters				
	Behaviour	*Disease*	*Mental satisfaction*	*Production rate*	*Longevity*
Effect	-	-	-	+	-

-, Negative effects on welfare. +, Positive effects on welfare

2.2.1.1 Nutritional factors

The difference between the main inputs and outputs of high and average yielding dairy cattle are demonstrated in Table 2.5.

Table 2.5 Main inputs and outputs of high and average yielding dairy cows (Adapted from DRC, 2000)

	Average yielding cows*	High yielding cows**	Change for high yielding cows (%)
Inputs			
Feed intake (kg DM d^{-1})	17.9	25.3	↑ 41
Water consumed (litres d^{-1})	50	107	↑114
Outputs			
Milk yield (kg d^{-1})	32	48	↑ 50
Milk protein content (%)	2.88	2.96	↑ 3
Milk fat content (%)	4.02	3.31	↓ 18
Liveweight (kg)	541	670	↑ 24

**Average-yielders: Holstein (30%) Friesian. **High yielders: Holstein (85%) Friesian.*
(Both groups were in 3rd and 4th parity, 12 weeks post-calving & fed a total mixed ration based on maize silage)

From Table 2.5, the major differences in feed and water intake between the two groups of cows can be seen. This reinforces the need to provide increased inputs to

meet the additional requirements of the high yielding cow. In addition, an intensive management system is required to satisfactorily cater for these needs.

It is also important to consider the challenge of lactation that is faced by the high yielding cow (Fig. 2.3). Milk yield gradually increases from calving, peaks at approximately 6 weeks post-calving and then steadily declines until the cow is dried off (Phillips, 2000b). In early lactation, appetite lags behind milk production and peaks at approximately 10-12 weeks post-calving (English *et al.*, 1995). Appetite, and therefore food intake, is reduced as the calf grows in late lactation.

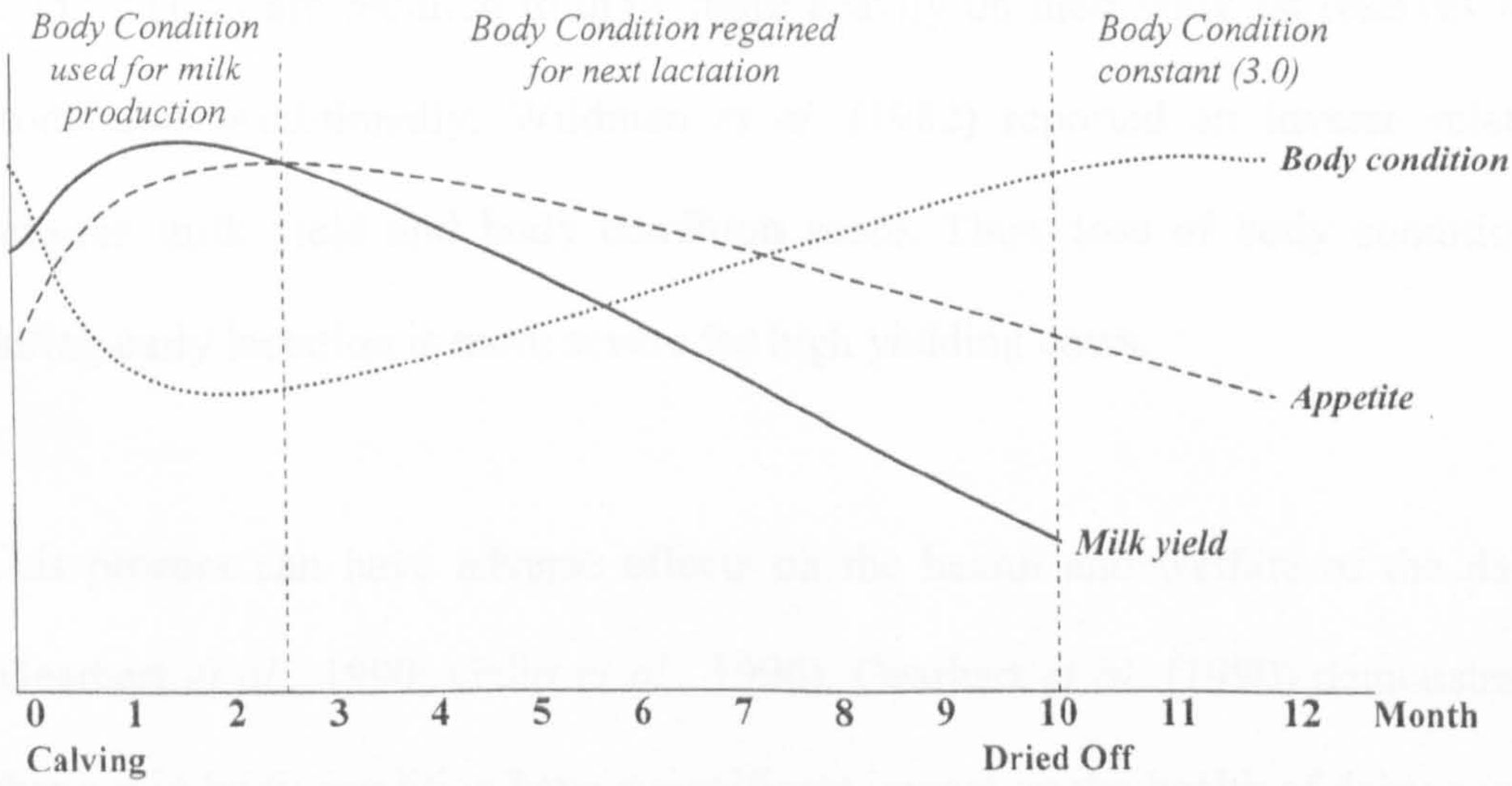


Fig. 2.3 Typical changes in body condition, appetite and milk yield during lactation for a mature cow (Adapted from English *et al.*, 1995)

The cow has to draw on body fat reserves in early lactation in order to satisfy the nutrient requirements of milk production. This results in a loss of body condition. Veerkamp *et al.* (1994) observed that high yielding dairy cows have a significantly lower mean body condition score. In addition, the high yielding cow undergoes a

more prolonged (and higher) post partum loss of body condition than lower yielding dairy cows (Gallo *et al.*, 1996). This occurs because dairy cows supply more than 30% of their energy reserves to milk production in early lactation and body fat mobilisation can continue until milk yield is less than 80% of the peak yield (Bauman & Currie, 1980). Thus, high yielding cows are at a greater risk as they tend to partition more of their nutrients to milk and less to body reserves in comparison with low yielders. This can result in severe negative energy balances and emaciation.

The energy balances of high yielding dairy cows are lower up to 11 weeks post-partum in comparison to lower yielding cows (Harrison *et al.*, 1990). Therefore high yielding cows are required to draw more heavily on their body fat reserves for milk production. Additionally, Wildman *et al.* (1982) reported an inverse relationship between milk yield and body condition score. Thus, loss of body condition score during early lactation is more severe for high yielding cows.

This process can have adverse effects on the health and welfare of the dairy cow (Gearhart *et al.*, 1990; Gallo *et al.*, 1996). Gearhart *et al.* (1990) demonstrated that changes in body condition have a significant impact on the health of dairy cows. They observed that underconditioned cows (body condition score < 2) are more susceptible to an increased chance of mortality, dystocia, and foot problems post-partum compared with cows in good body condition (body condition score $\geqslant 3$).

Webster (1993a) compared the energy requirements of the dairy cow for milk production with those of other lactating species. For example, in comparison with the lactating sow or bitch, it is not especially high. It is not the intensity of lactation

however that is potentially damaging to the animal's welfare, but the duration. Webster (1993a) suggested that 'the severity of suffering experienced by an individual animal is defined by its intensity and by its duration.' Webster (1993a) expresses this as: Severity of suffering = f_1 (intensity) x f_2 (duration).

It is evident then, that the high yielding cow is under considerable stress throughout her productive life and especially during the early stage of milk production (Webster, 1987 & 1993a; English *et al.*, 1995; FAWC, 1997). This can result in the onset of production diseases such as hypocalcaemia, hypomagnesaemia, acetonaemia and Fatty Liver Syndrome (Webster, 1993a).

All of these metabolic disorders impact on the cows' welfare and productive efficiency and can, at worst, result in natural death or premature culling.

2.2.1.2 Health problems

Hypocalcaemia is most likely to occur during early lactation (English *et al.*, 1995). The cow has a large store of calcium in her bones and the food in her intestine, but only has a small amount of readily available calcium circulating in her blood (English *et al.*, 1995). This is not enough to meet the sudden demand required for milk production post-calving. At this stage (*i.e.* early lactation) she has to produce five times the amount of calcium that was being supplied to the calf in late pregnancy (English *et al.*, 1995). This causes a reduced concentration of calcium in the blood, resulting in the condition commonly referred to as milk fever. The cow becomes lethargic, unsteady and has difficulty rising due to paralysis of the hindquarters (English *et al.*, 1995).

Hypomagnesaemia is generally a seasonal problem for the grazing dairy cow (English *et al.*, 1995). At certain times of the year (mainly spring) grass magnesium concentrations are too low to provide the cow with her daily requirement [as the cow is unable to store magnesium in her body (English *et al.* 1995)]. This gives rise to low blood concentrations of magnesium and causes excitement, unsteadiness and fitting, which is accompanied by frothing at the mouth (Boden, 1995). The high yielding cow loses more magnesium due to her increased level of milk production as milk magnesium is constant and she can not regulate its output (Arney, 2000). This makes her more susceptible to hypomagnesaemia.

Acetonaemia is caused by the cow drawing extensively on her body fat reserves to provide her with enough energy to sustain the high levels of milk production in early lactation (English *et al.*, 1995). As the cow's body fat reserves are utilised, ketones (toxic chemicals) are produced, which can be detected by smelling the cow's breath as this contains acetone (ketone bodies) and smells sweet (like pear drops) (English *et al.*, 1995). The cow becomes dull and lethargic. This condition can be prevented by ensuring the cow is not too fat (body condition score $\leqslant 3.5$) in late pregnancy, since a thinner cow will have a greater appetite and therefore will consume enough energy to sustain her high milk yield potential. Consequently, she will be less likely to draw on her body fat reserves to make up the energy deficit. Additional measures should also be taken to maximise feed intake levels in early lactation by providing a well balanced, high-energy diet. Acetonaemia can lead to fatty liver syndrome. .

Fatty liver syndrome is caused by the same factors governing the onset of acetonaemia (English *et al.*, 1995). The mobilised fat is stored in the liver prior to

being broken down. Too much fat in the liver affects its functioning and can cause liver failure. This further depresses the appetite of the cow and she becomes lethargic (English *et al.*, 1995). Ensuring the cow receives a well balanced diet can prevent this.

2.2.1.3 Behavioural consequences

In addition to the metabolic problems outlined above, the cow's welfare may also be affected by the sensation of hunger. This is mainly due to the feed intake capacity of dairy cows not being increased to the same extent as milk production (Kamphues, 1998). This infers that dairy cows, especially high yielders, cannot consume sufficient energy to satisfy their appetite. It is the grazing cow that is at a higher risk of suffering from hunger than those cows managed under intensive conditions. Phillips (1998) noted that the most variable grazing period for dairy cows is at night. This is determined, in part, by the cow's milk yield whereby high yielders take a 'midnight snack'. It is unusual for cows to graze at night due to a vestigial fear of predators (Phillips, 1998) or difficulties in herbage selection (Phillips & Hecheimi, 1989). This suggests that high yielding cows have a high motivation to graze at this time, which is likely to stem from an increased appetite and the feeling of hunger (Phillips, 1993). This seems a reasonable suggestion as 'cattle are motivated to feed by hunger, which is alleviated by the feeling of satisfaction or satiation' (Phillips, 1993).

A particular problem for the grazing cow is the low rate of grass intake. A typical dairy cow consumes approximately 15-20g DM min^{-1} as compared with 40g DM min^{-1} for silage (Phillips, 2001). Therefore, extensively managed high yielders may have to graze for longer as they do not have enough time to consume enough grass (Phillips & Denne, 1988). They may achieve this by grazing earlier in the morning (Phillips &

Rind, 2002) and later at night in comparison with their lower yielding counterparts (Phillips & Denne, 1988). These coping strategies may result in the cow having to neglect the expression of important behaviours such as lying down to rest (Phillips, 1993). As cattle demonstrate a strong motivation to lie down (Metz, 1985) an extended grazing period may be indicative of the severity of hunger being experienced by the cow.

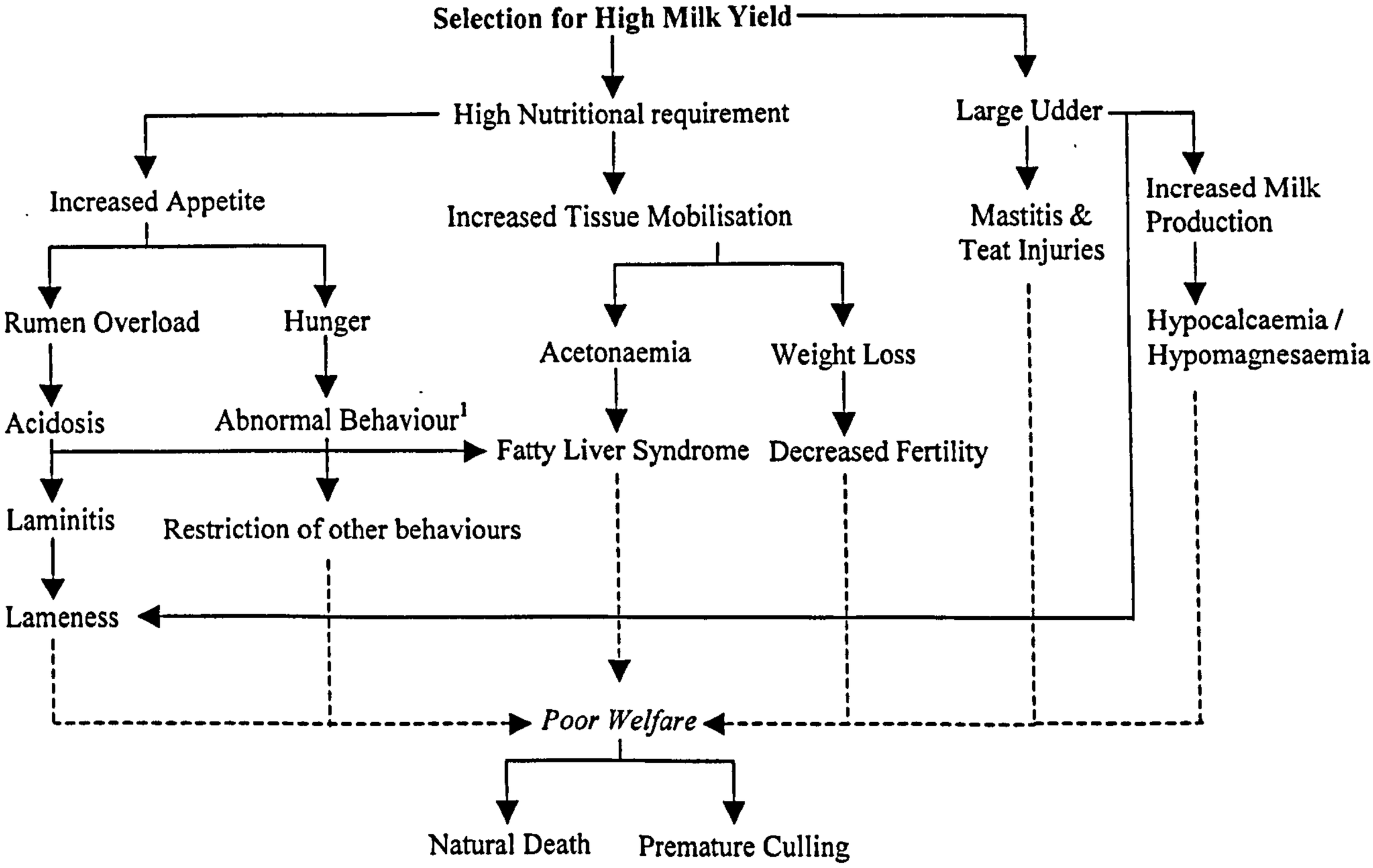
In conclusion, Webster (1993a) has pointed out that high yielding grazing dairy cows may feel, simultaneously, 'hungry, full-up and physically tired'. Hungry because they are unable to consume enough energy to meet their genetically determined high level of milk production; full-up because they are consuming bulky food, which is slowly digestible; and physically tired, because of the intensity and duration of milk production and due to the conflicting needs of grazing and rest.

To overcome these problems cows have been fed a balanced high energy ration to complement their yield. This requires intensive management however, which, due to its nature, restricts the cow's behaviour thereby compromising her welfare. As suggested by Blakeway (1998) 'clearly, for good welfare, a high producing cow requires a higher and more consistent level of husbandry and nutrition than does a cow less highly selected for milk production.' Nevertheless, Phillips and Leaver (1985a) have suggested a solution to this problem by suggesting a system involving grazing with additional forage supplementation. This allows behaviour to be expressed relatively unrestricted and an adequate nutrient intake to be achieved.

2.2.1.4 Structural problems

Most of the health and welfare problems discussed above result from a nutritional deficit, *i.e.* the cow being unable to obtain enough nutrients to sustain a high level of milk production. However, selecting for increased milk yields inevitably results in bigger cows that have larger udders (Webster, 1993a) (Fig. 2.4), which also causes problems for the high yielder. Apart from being uncomfortable, a large udder can also prevent the cow from walking normally. The cow is forced to walk with a gait which puts extra pressure on one side of the hoof which may cause solar ulcers and lameness (Webster, 1987). This can result in early culling due to an excessive loss of weight, reduced milk yields, and reduced fertility (FAWC, 1997). Lameness is a painful disease and consequently interferes with the cow's locomotion, ability to stand for long periods and the performance of other behaviours (Ward, 2001). Hassall (1993) conducted an experiment to investigate the implications of lameness on cattle behaviour. The results indicated that lame cattle spent more time lying (to release the pressure from standing), and less time feeding, possibly to avoid competitive interactions with others.

To counteract many of the problems discussed in this review it is necessary to provide the high yielder with a balanced diet that should be fed according to yield, lactation stage and body condition. This will help reduce weight loss and ensure health and fitness is maintained (FAWC, 1997).



[1]*Behaviour, which differs in pattern, frequency or context from that which is shown by most members of the species in conditions which allow a full range of behaviour (Fraser & Broom, 1997). E.g. a longer time spent grazing.*

Fig. 2.4 Health problems impacting on the welfare of the high yielding dairy cow (Adapted from Webster, 1993a)

In summary, we can use the Five Freedoms (FAWC, 1997) (Table 2.1) to identify the major factors contributing to the poor welfare of the high yielder (Adapted from Webster, 1993a):

1. **Hunger** due to a failure to supply and/or consume nutrients appropriate to the genetic and physiological potential of the cow.
2. **Discomfort** due to loss of body condition, a large udder and infrequent milking.

3. **Pain** and restricted movement primarily due to an enlarged udder and its consequent problems; Occurrence of metabolic diseases, and an increased susceptibility to infectious disease due to immunosuppression caused by metabolic exhaustion.
4. **Difficulty in expressing normal behavioural patterns** due to distortion of body shape, an intensive environment and the possible time conflict between grazing and the performance of other behaviours.
5. **Distress** due to the factors outlined above and as a result of prolonged high production.

2.3 Assessing welfare

Assessing welfare is difficult, as it cannot be carried out directly, *i.e.* we do not have access to the private experiences of animals. Wechsler *et al.* (1997) stated that, '...animal welfare is assessed by means of indicators that are assumed to be related to the subjective feelings of animals.' Therefore by means of measuring psychological, physiological and behavioural parameters we can attempt to assess what an animal is experiencing (Sandoe and Simonsen, 1992). English and Edwards (1992) have suggested a number of such indicators. These are:

- Productivity – *e.g. Growth rate; Food conversion efficiency; Yields; Conception rates*
- Mortality and Health – *e.g. Cuts; Bruises; Lesions*
- Ethological Measures – *e.g. Abnormal behaviour; Preference tests*
- Physiological Measures – *e.g. Heart rate; Presence of stress hormones*
- Immunological Measures – *e.g. Size of lymph nodes; Antibody production*

Supporting this, Tschanz (1987) noted that there are certain characteristics, which can be observed and measured, acting as useful indicators of welfare. Tschanz (1987) proposed that all animals aim to satisfy their requirements (*e.g.* food, water, shade) and avoid harm in order to enhance survival and reproduction. If an animal is prevented from achieving these functions, its ability to cope is impaired, and the resulting deficiencies (*e.g.* malnutrition, injury, abnormal behaviour) can be measured objectively. Each indicator is outlined briefly below:

Productivity: Poor welfare can lead to reduced levels of productivity. For example, individuals may not grow at the normal rate, may fail to come into oestrus, or may have reduced conception rates (English & Edwards, 1992). Improving welfare, by identifying and correcting the problem/s, can increase production efficiency. However, it is not sufficient to suggest that an animal is in a good state of welfare just because it is growing or producing well, the routine use of antibiotics and the feeding of a basic ration can produce satisfactory results (English & Edwards, 1992). In addition, performance levels are influenced by many factors within a system.

Mortality & Health: Although mortality is a 'black' or 'white' indicator of welfare, it is not a sensitive one (English & Edwards, 1992). A more sensitive measure is to look at health, which can be scaled, *i.e.* from very poor to very good. Health measurements include incidences of disease, skin lesions and lameness (Edwards *et al.*, 1985). Dawkins (1980) suggested that these are only of a welfare concern if the animal is experiencing unpleasant mental experiences as a result of them.

Ethology: Observing an animal's behaviour has been used for many years to determine the health of an animal. Well before clinical symptoms occur, modifications in behaviour may indicate that an animal is having difficulties in adapting to its environment (Wechsler *et al.*, 1997). Many diseases have been named after such observations, for example 'grass staggers' (in dairy cattle), 'wobbler' (in horses) and 'louping-ill' (various animals) (Boden, 1995). Similarly, behavioural observations can be a powerful tool in determining welfare. An animal's behaviour is an effect of its mental state (Dawkins, 1990). That is to say, for example, that if an animal is hungry, it seeks food; if it is cold, it seeks warmth. These behaviours can indicate what an animal is thinking or feeling. It is therefore possible, to some degree, to distinguish the subjective from the objective. Hence, as far as welfare is concerned, behavioural measurements may be the most powerful assessment tool scientists have. Through an accurate knowledge of behaviour it may be possible to understand something of the animals' world from its point-of-view (Dawkins, 1990).

Any changes in behaviour, which vary from normal, may indicate compromised welfare. These include activities such as vices, stereotypies and escape behaviours. Trying to define the term 'normal' is often difficult but may be considered as one which is similar in pattern, frequency or context to that which is shown by most members of the species in conditions which allow a full range of behaviour (Fraser & Broom, 1997).

A major problem with assessing behaviour is that of interpretation. The interpretation of results in some cases is based on the observer's opinion, as there is no scientific standard on which to base conclusions. This highlights the importance of having an

accurate and comprehensive knowledge of the behaviour of the animal being studied (relative to the study being carried out). For example, if studying fear in chickens it would be appropriate to have a knowledge of what that fear response looks like. Mason and Mendl (1993) proposed that a plausible way of finding measurable changes in an animal's behaviour, which is reliably correlated with its subjective feelings, is to subject animals to 'stressors' (stimuli scientists feel must be unpleasant, for example, hunger) and then read the animal's behaviour.

Physiology: When an animal is stressed, a series of physiological processes take place. The origin of these processes is in the hypothalamus region of the brain (Smidt-Nielson, 1983). This triggers two paths of action: a nervous pathway (the 'quick' response) and the hormonal pathway (the 'sustained' response) (Smidt-Nielson, 1983). Both routes result in the activation of the adrenal gland (Fig. 2.5) that brings about the "fight" or "flight" response seen in animals. Heart rate and respiration will increase as the level of stress hormones in the blood plasma increase (English & Edwards, 1992). There are problems associated with measuring these responses. For example, the methods used can be invasive and stressful (English & Edwards, 1992). Handling and restraint of the animal may also be necessary, which can also cause added stress. Another disadvantage is that stress hormone production varies considerably between individuals (English & Edwards, 1992). In addition, hormone release is pulsatile, and shows a circadian rhythm, so that results will vary depending on the time of day they were taken. From these problems, less disruptive methods of assessment have evolved. These include indwelling catheters and the use of remote blood sampling equipment (such as developed by the Macaulay Land Use Research Institute), which reduce the stress associated with taking measurements.

Another method used is the ACTH (Adrenocorticotrophin hormone) challenge test. The ACTH challenge test is used as a method of discovering the activity of the adrenal cortical enzymes (Fraser & Broom, 1997). An animal that is subject to stress for a long period will use its adrenal cortex more frequently to produce stress hormones called corticosteroids. This means its cortical enzymes are likely to be upregulated due to a hyperactive adrenal gland (English & Edwards, 1992). ACTH stimulates the adrenal cortex to produce the stress hormone cortisol (Fig. 2.5). Thus, an injection of a large dose of ACTH will stimulate the adrenal cortex to produce the maximum amount of cortisol. The amount produced is indicative of the level and duration of stress an animal has experienced, or is experiencing (Fraser & Broom, 1997).

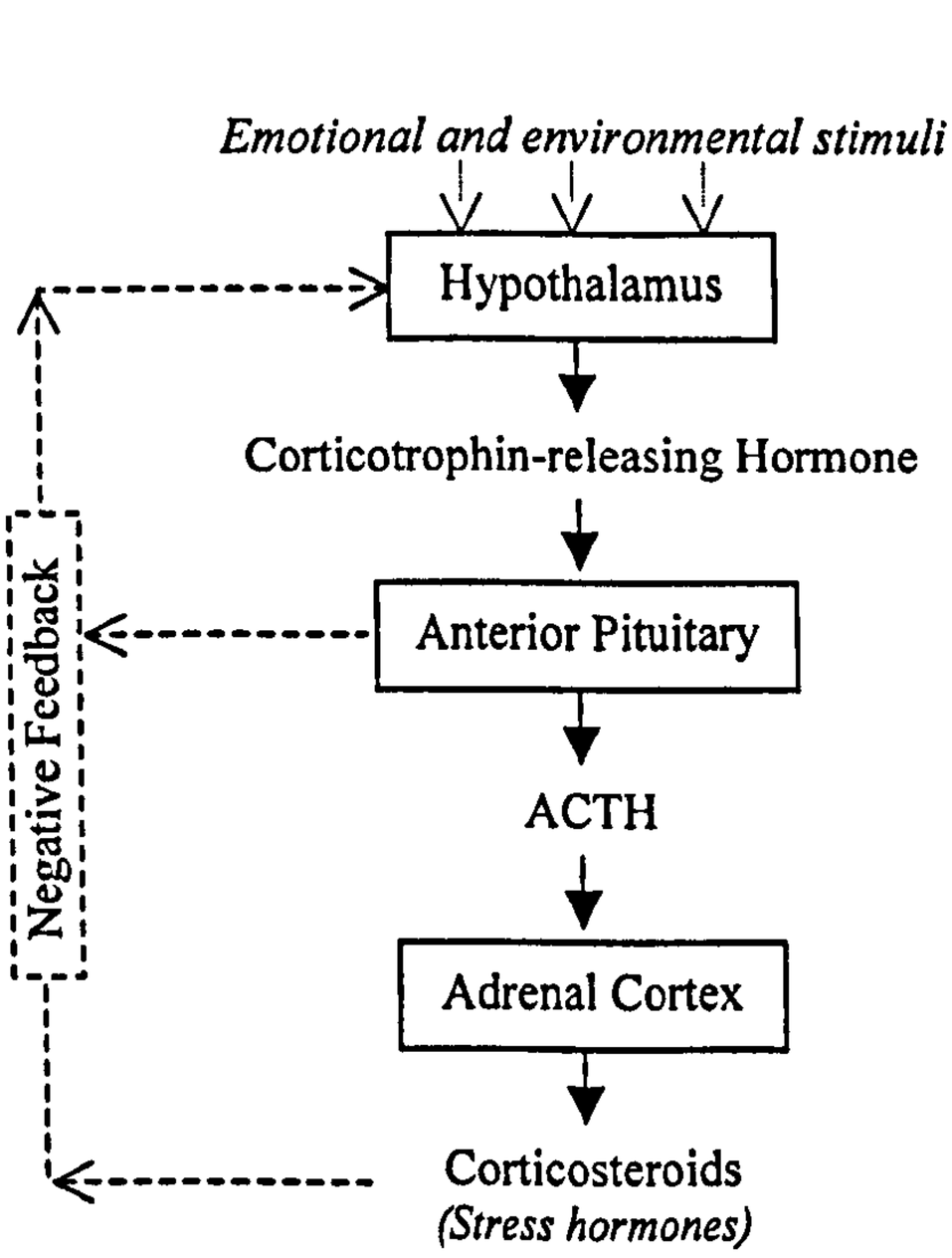


Fig 2.5 Hypothalamo-pituitary-adrenocortical (HPA) axis (Smidt-Nielson, 1983)

Immunology: Immunological methods provide an insight into long-term responses. For this reason, they are useful because the measurement process does not affect them. Environmental stressors can increase an animal's susceptibility to disease (English and Edwards, 1992). This is caused through stress acting on the immune system (Kelley, 1980). For instance, the stress hormone cortisol can reduce the size of lymph nodes, proliferation of the lymphocytes (Kelley, 1988) and the number of antibodies produced (Martin, 1987). This results in an impaired resistance to infection and therefore a higher susceptibility to disease.

Argument of analogy: The aim of assessing welfare it to obtain some idea of how an animal is feeling. However some of the welfare indicators suggested above do not provide us with this insight, so other means have to be sought. Sandoe and Simonsen (1992) suggested that we may be able to understand how an animal is feeling by using the 'argument of analogy'. This is suggesting that by knowing how we feel ourselves in certain situations, we can, in part, attempt to understand the feelings of other animals in similar situations, *i.e.* draw an analogy between non-human animals and ourselves. This is not to say we are being anthropomorphic. Through rational argument based on scientific literature it is possible to follow a route of logical reasoning. For example, higher animals have similar nervous systems and physiological mechanisms to our own that function in the same way. This suggests that they transmit, receive and therefore interpret these signals similarly. This thought is shared by Brambell (1965) who stated that '..there are sound anatomical and physiological grounds for accepting that domestic mammals and birds experience the same kind of sensations as we (humans) do...' Drewett (1983) has also pointed out that '..we are not just *like* animals, we *are* animals. We are mammals and we share

many psychological features with them.... In the case of fear, it seems to me beyond any reasonable doubt that we do share fear responses with many other species.' This does not suggest that the subjective life of animals is equal to that of humans. Nevertheless, it is similar and varies only according to its evolutionary stage (Wemelsfelder, 1997). Therefore, their subjective experiences may not be to the same level as ours, but they do posses them. This theory has been argued against on the grounds that humans are able to verbally communicate their fears. However, psychiatrists who work on fear in humans know that what humans say gives limited direct access to how they feel (Drewett, 1983). Instead they use methods such as hormone responses, blood pressure, stimulus avoidance and behaviour to evaluate fear (Drewett, 1983). These are all non-verbal cues. This evidence does not suggest that the stimuli eliciting a fear response in ourselves will also bring about the same response in, for example, a turkey. But it suggests that there are elements within a fear situation that will bring about a fear response in both human and non-human animals. It is these common elements that should be determined. Even though human and animal welfare does not have the same specific content, it may share certain common elements (Sandoe, 1996). It is therefore possible to determine fear in animals (*i.e.* how animals feel) by comparison to fear experienced in ourselves. Additionally, using the argument of analogy, it is possible to record the behavioural changes that occur in humans who feel, for example, scared, worried or in pain, and look for similar changes in those animals' whose welfare we are trying to assess (Mason & Mendl, 1993). From this rationale it may be possible to determine other feelings such as boredom and apathy in non-human animals if we can uncover the common elements that affect them and us. Sandoe and Simonsen (1992) suggested that '..by means of analogies it may be argued that animal welfare consists of subjective experiences such

as pain, boredom, pleasure and expectation'. These analogies should not be specific but of an abstract, elementary nature, which will allow them to be applied to all sentient animals (Sandoe & Simonsen, 1992). In a sense this is already practiced as we have a general concept of welfare to cover all sentient animals, *i.e.* what is good for a cow may not be good for a pig – but it is the welfare principles that matter.

In conclusion, it is not advisable to use any single measurement to assess the welfare of an animal. Each method has advantages and disadvantages to its use. Some are limited in their application and may be affected by several other factors. Considering this, it is best to use a combination of techniques to make a successful and accurate welfare decision. Finally, welfare assessment, whilst it should be scientifically based, ultimately involves an ethical judgement.

2.3.1 Behavioural measurements of animal welfare

2.3.1.1 Relationship between subjective feelings and behaviour

The main aim of welfare assessment is to obtain some idea of how an animal is feeling (Dawkins, 1990). For this to be possible, it is necessary to have an objective measurement that is related to the animals' subjective experiences. A number of researchers (Dawkins, 1990; Broom & Johnson, 1993; Mench & Mason, 1997; Broom, 1998) have suggested that the study of behaviour provides the best measurement for gaining access to the private experiences of animals'. However, this assessment technique assumes that a subjective/objective relationship exists, *i.e.* there is a link between the subjective experiences of an animal and the way it behaves. Wemelsfelder (1997) stated that both behaviour and subjective experience 'form an

integrated, dynamic, expressive whole'. This is to say that behaviour is an outward display of the subjective state of an animal. In the words of Wemelsfelder (1999):

> "We do not just see 'behaviour', but, over and above that what we see is a 'behaver', a dynamic agent. We do not merely see sitting, walking, or licking going on, we see an animal who sits, walks or licks. It is not the legs that walk, the tongue that licks; it is the animal who walks with its legs or licks with its tongue."

The sceptic may suggest that one's inner world is uniquely private and so how is it possible to tell what another individual is feeling or experiencing. It is true that our own experiences and feelings are personal and unique, but this does not imply that they are inaccessible and wholly private (Wemelsfelder, 1997). Because it may not be possible to directly experience the feelings of another individual this does not mean we have no access to them (Wemelsfelder, 1997).

Behaviour has evolved to aid the survival of an animal (Moss, 1992). Animals use their behaviour to adapt themselves to their environment (Martin & Bateson, 1995) and they behave according to, and in order to satisfy, their needs (Tschanz, 1987). Thirst and hunger, for example, are negative subjective experiences and drive the animal to modify its behaviour to satisfy its requirements and therefore relieve or reduce those negative feelings. It is these needs that alter motivational states, which in turn alters the animal's behaviour (Fraser & Broom, 1990). Mench and Mason (1997) stated that behaviour "is what animals do to change and control their environment, and thus provides information about their needs, preferences and internal states". This

implies that through an understanding of behaviour it is possible to determine when an animal is frightened, ill, in pain and so on (Mench & Mason, 1997). For example, an animal may try to escape, avoid something, become immobile, aggressive or vocalise. In addition, a sick animal, for example, may drink less water, eat less, cease grooming activity and become less active (Hart, 1988). These actions can indicate how an animal is feeling. It is the display as well as the absence of behaviour that can be used for determining an animal's welfare. This project will assume that there is a subjectivity/objectivity relationship.

2.3.1.2 Relationship between behaviour and welfare

Veterinarians can use behavioural measures to help assess the health status of animals. This is because the behaviour of an animal can inform the vet, to some degree, of how it is feeling. Fraser and Broom (1997) stated 'altered behaviour is usually the first indication of illness,' for example, an eye that is very mobile and exposed suggests anxiety, while a fixed stare may indicate stress. The health of an animal impacts on the way it feels and an animal will alter its behaviour depending on how it is feeling. As welfare is based on feelings, behavioural observations provide a valuable insight into determining this. As an example, a lame cow will feel uncomfortable by putting pressure on her diseased limb. She will therefore alter her locomotion accordingly to relieve the amount of pressure applied. From observing this behaviour we can suggest that she is experiencing some degree of pain and so her welfare is being compromised (Fig. 2.6).

In Fig 2.6 it is health (the stimulus) that is impacting on the animal's welfare (*i.e.* feelings) and therefore determining its behaviour.

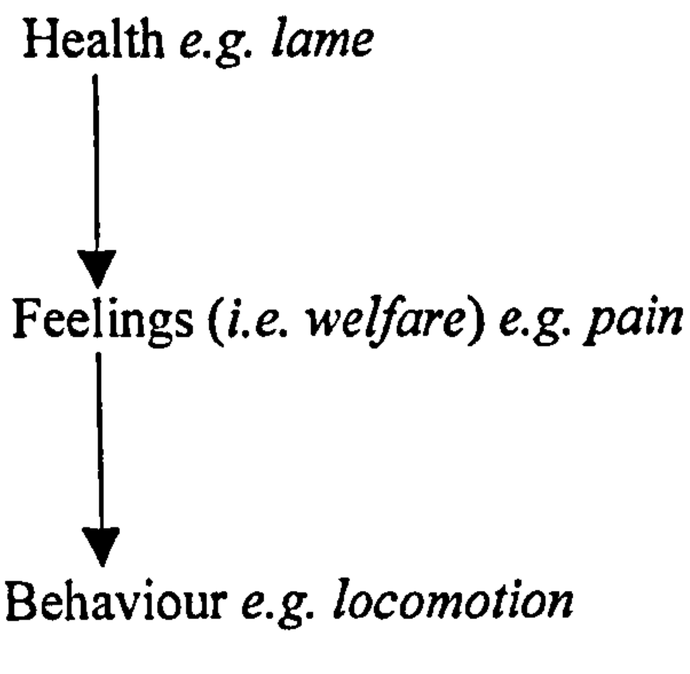


Fig. 2.6 The relationship between welfare (*i.e.* how an animal feels) and behaviour (NB arrow denotes direction of impact).

2.3.1.3 Relative merits of behavioural measurements of animal welfare

It can be suggested that behavioural measurements, correctly interpreted, can be used to provide a relatively accurate assessment of an animal's welfare. However, it does have certain limitations and drawbacks. It may be difficult to diagnose the specific feeling being experienced by an animal. This is because the display of a certain behaviour (or behavioural pattern) may be caused by more than one factor or feeling. For example, an animal may become more aggressive because it is frustrated, bored or in pain (Mench & Mason, 1997). So there may be more than one factor that induces the same behavioural expression. Nevertheless, the expression of such behaviours are indicative of a reduced level of welfare, and it may not be necessary to identify the precise feeling being experienced.

It may be possible to determine the specific emotions being experienced by an animal by looking at the situation it is in. If the stimuli acting on the animal can be identified, it may be possible to determine the animal's feelings by using the 'argument of analogy'. It is possible to do this because it is the stimuli within the animal's

environment that influence its feelings and its feelings are expressed behaviourally. For example, a chicken that flaps, vocalises and displays escape behaviours when a human is present is likely to be experiencing some level of fear (Fig. 2.7).

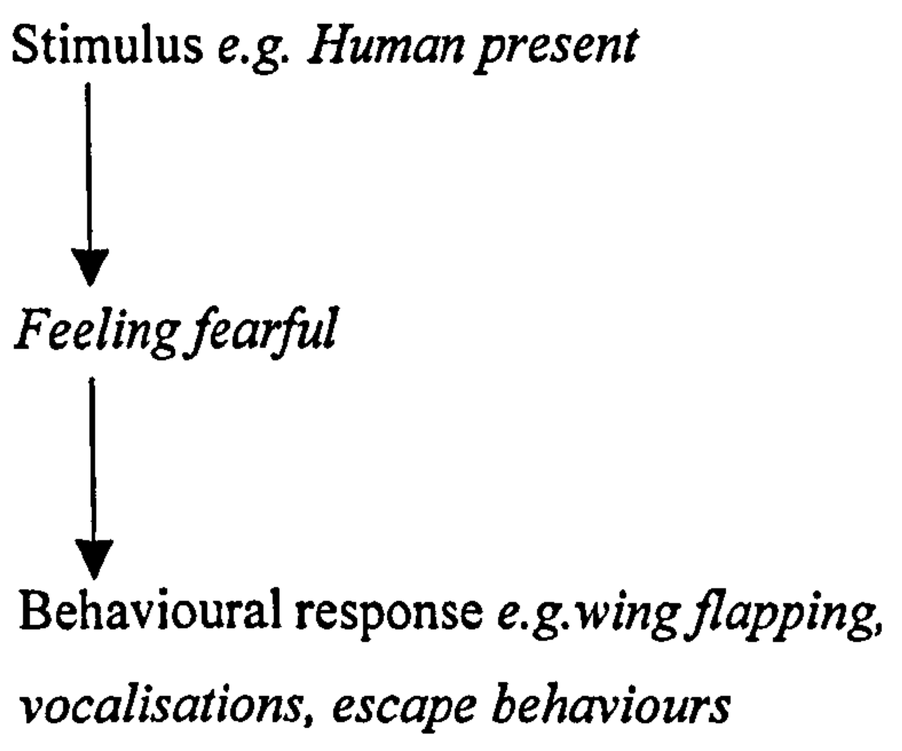


Fig. 2.7 An animal's feelings may be determined by identifying the influential factors affecting its behaviour and applying the 'argument of analogy'.

Another problem is that individuals within the same population may react differently to the same stimulus and therefore feeling. For example, in response to a fear situation some humans may freeze whilst others may flee; some may scream whilst others remain silent. However, it could be argued that both behaviours, however different, are indicative of a fear response.

Broom and Johnson (1993) have also suggested that 'some behavioural responses are not related in intensity to the degree of difficulty encountered, but are all-or-nothing.'

Broom (1993) suggested that welfare can be measured on a scale from good to poor. The same author states that due to this it is possible to determine the level of welfare an animal is experiencing in accordance to its position on the scale. This can be

achieved because an animal will vary its level of response depending on the severity of the problem encountered. As time proceeds the severity of the problem increases, the level of welfare decreases and the level of (*e.g.* behavioural) response changes. For example, a sow that is confined will show various levels of response that increase in severity with time (Fig. 2.8). This is indicative of a gradual decline in welfare (Broom, 1993).

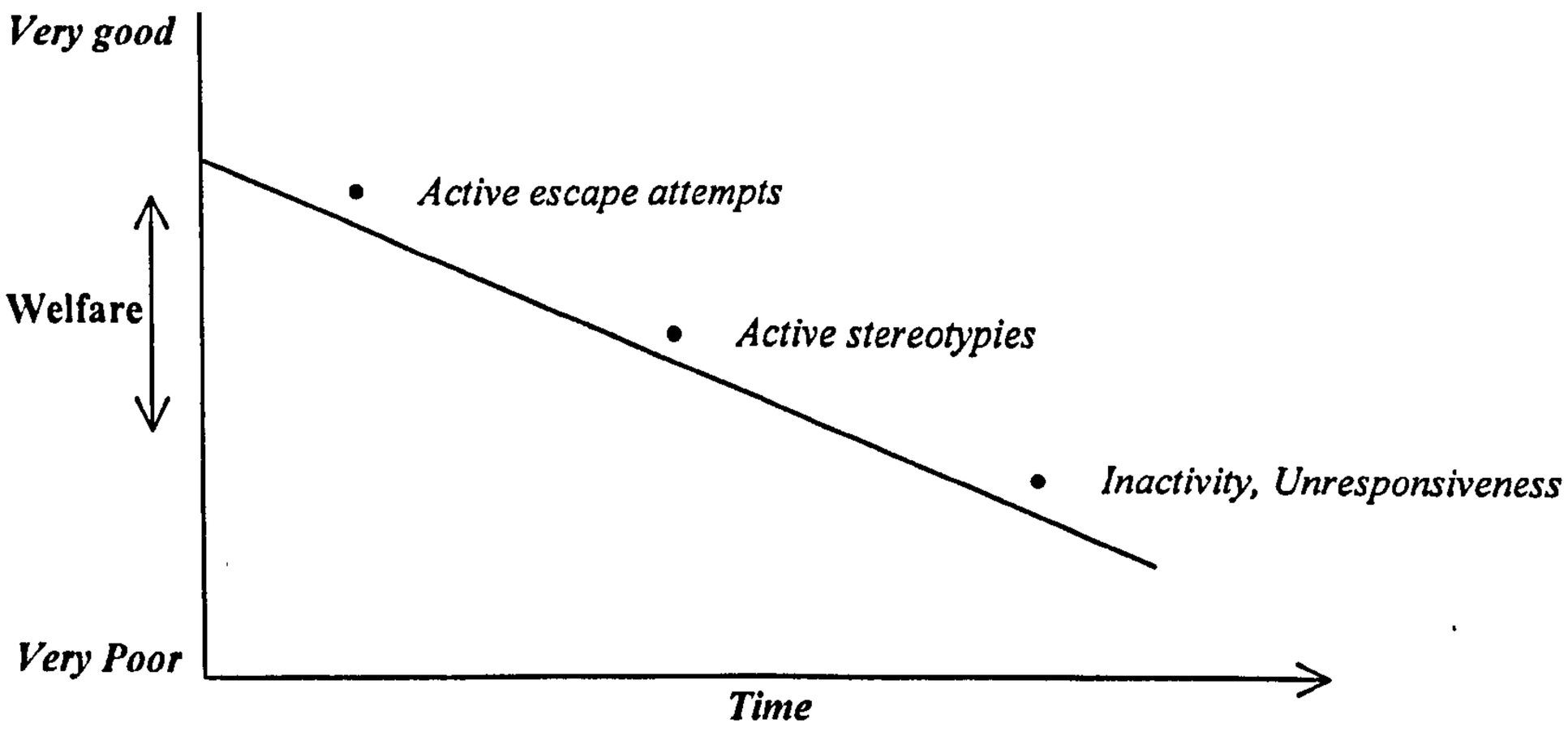


Fig. 2.8 The welfare continuum of the various responses of sows to close confinement over a period of time (Adapted from Broom, 1993)

The welfare indices used in Fig. 2.8 are all based on behavioural observations. This indicates the significance of using behavioural parameters in welfare assessment. They provide the observer with visual signals of emotional content. In addition, they provide a scale indicating the severity of suffering being experienced by the animal, *i.e.* from very good to very poor. The above concept can be applied to provide some indication of the level of welfare (the subjective experience of hunger in this project) being experienced by the high yielding dairy cow (Fig. 2.9).

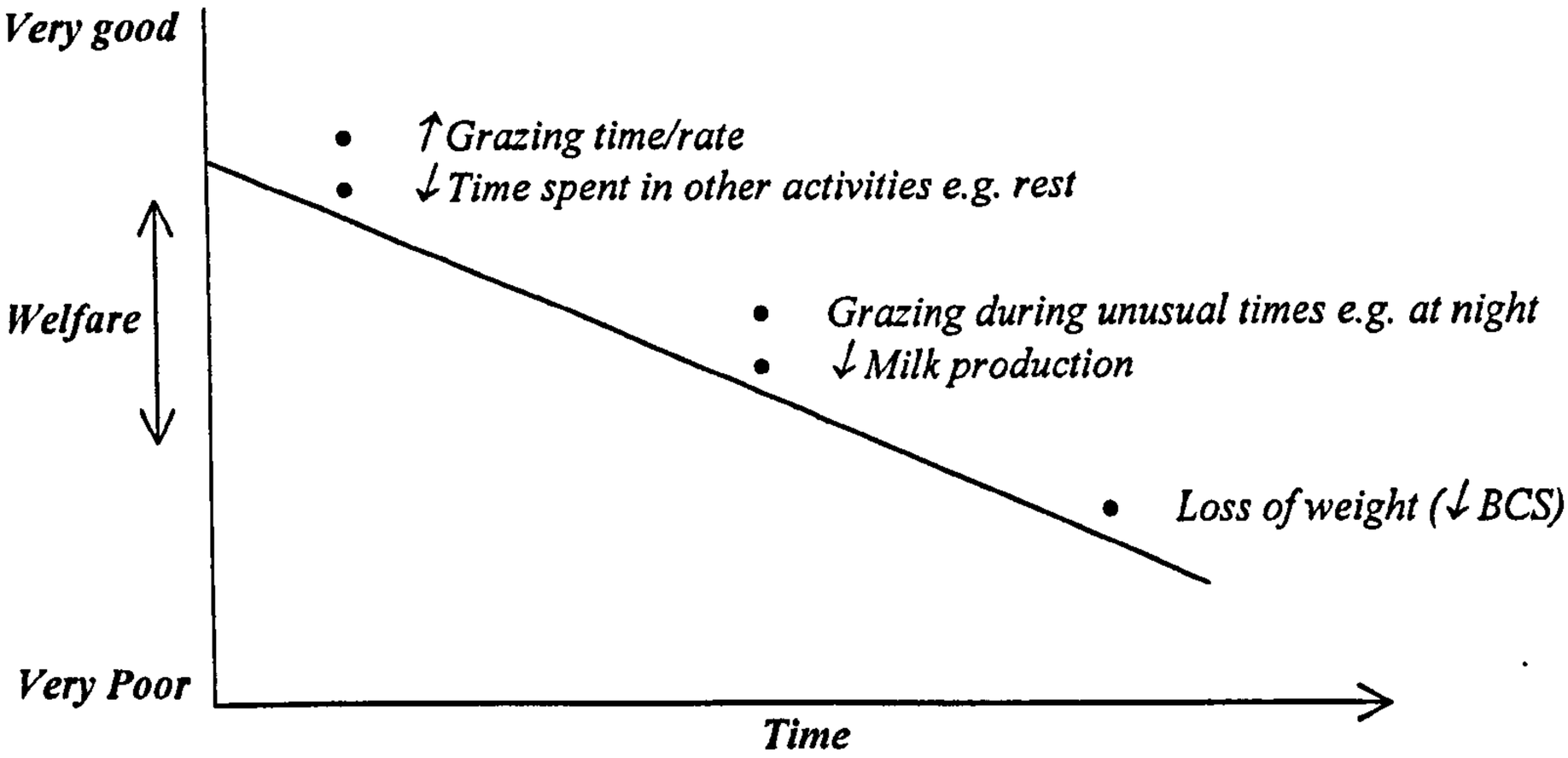


Fig. 2.9 A welfare continuum of the various responses of high yielding dairy cows to hunger over a period of time (Adapted from Broom, 1993)

2.3.1.4 Methods of assessment

The Five Freedoms represent a framework for welfare assessment (Table 2.1). Given this, the importance of behaviour as an assessment tool for welfare is recognised (Gonyou, 1994).

In order to successfully interpret an animal's behaviour it is important to have a detailed knowledge of that species' behaviour, *i.e.* its species-specific behaviour. This can be achieved by developing an ethogram for that species, which is carried out in conditions that allow a full range of behaviours. This provides a baseline of behaviours to which other members of the species can be compared. Duncan (1980) suggests that there are three ways that behavioural parameters can be used to assess welfare:

1. By looking for unusual or inappropriate behavioural changes.
2. By providing animals with choices on the assumption that they will choose what is best for their welfare.

3. By subjecting animals to stress situations, observing their behaviour and using this as an indicator of a standard response to the stress situation.

A popular method for assessing poor welfare is to look for abnormal behaviours, *i.e.* a statistically rare or different behaviour from that of a chosen population (Mench and Mason, 1997). If an animal is unable to cope with its environment using its normal range of behaviours, it may adopt abnormal behaviour patterns to achieve this (Table 2.6). Broom and Johnson (1993) state that those behaviours that indicate [poor] welfare are usually brought about due to the animal's attempts to cope with its environment. These can be, for example, stereotypies, which are typically seen in animals that are housed in confined environments. It is usually a mechanism employed by the animal to cope with boredom or frustration (Barnett & Hemsworth, 1990). Observation of such behaviours can therefore give some indication of how the animal is feeling.

As can be seen from Table 2.6 there are several types of abnormal behaviours, which are used to indicate poor welfare. Each category proposes that the animal is suffering to some degree. However, each category can indicate a different level of suffering being experienced by the animal. From this, it may be possible to suggest that an animal's welfare is more or less poor than another's by the type of abnormal behaviour it is displaying. It is also possible to put each behavioural category on a scale of its own to indicate the severity or degree of suffering being experienced by an animal (Fig. 2.10).

Table 2.6 Abnormal behaviours used to indicate poor welfare (Weipkema, 1983)

Behavioural Category	Definition	Examples
Detrimental Behaviours	Behaviours that cause injury to the individual itself or others	Tail biting and Feather pecking
Stereotypies	A repeated, relatively invariate sequence of movements which has no obvious purpose (Fraser & Broom, 1997)	Pacing; Rocking; Swaying; Pawing; Self grooming
Sham Behaviours	Behaviours performed in the absence of adequate substrate or environmental stimuli	Sham-chewing and Sham-dust bathing
Apathetic Behaviours	A reduced attentiveness towards external stimuli (Weipkema, 1983)	Motionless sitting
Re-directed Behaviours	Behaviour directed at an inadequate/abnormal object (Weipkema, 1983)	Excessive licking; Wind Sucking; Bar biting

Some scientists argue that changes in behaviour indicate that animals are adaptable creatures (Hughes, 1978). In the case of abnormal behaviours they suggest the animal is displaying that it has appropriately adapted to suit its environment thus indicating the absence of a welfare problem.

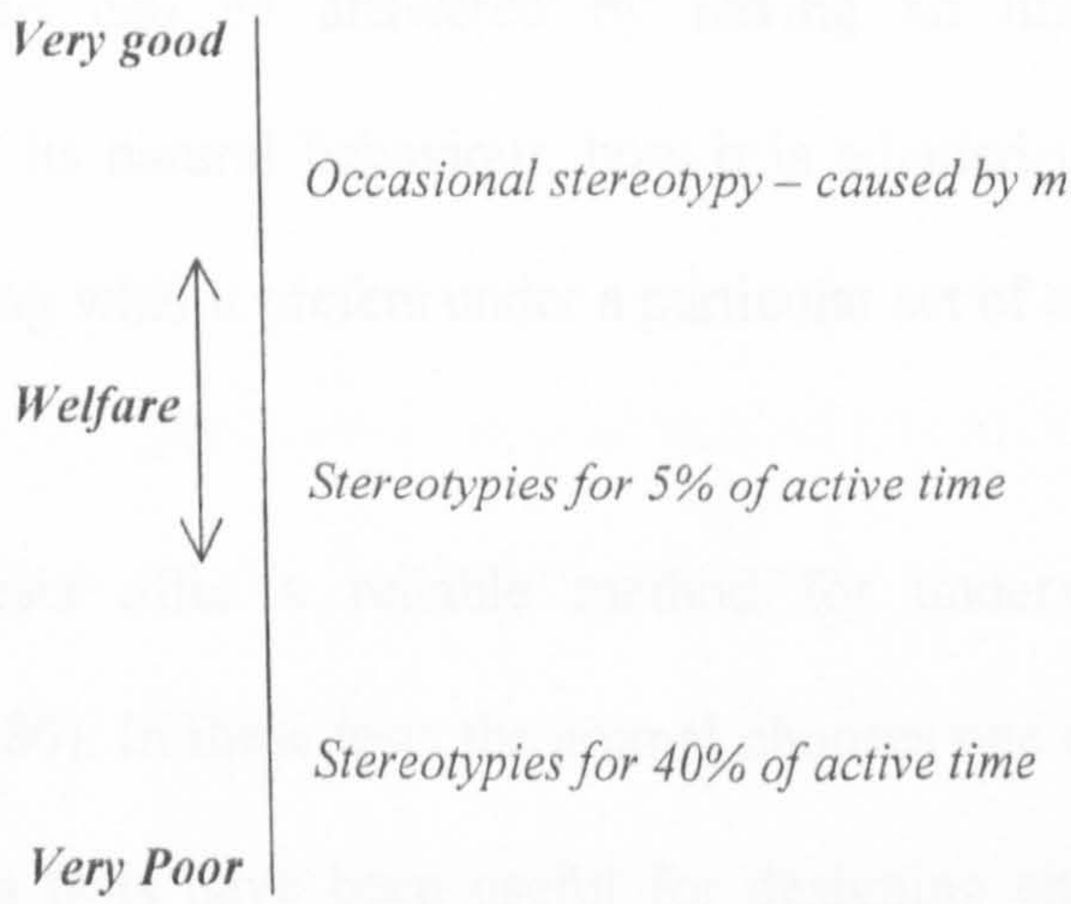


Fig. 2.10 A scale to link the frequency with which stereotypies are performed to the level of welfare being experienced by an animal (Broom & Johnson, 1993)

In conclusion, behaviour has been used extensively in attempts to determine how animals feel. As Wemelsfelder (1997) states "subjective experiences in animals' are in fact open to empirical observation, and as such are liable to objective analysis." It is primarily abnormal or deleterious behaviours that are used as indicators of poor welfare (Gonyou, 1994). Communication, such as vocalisations have also provided information regarding the subjective experiences of animals (Watts & Stookey, 2000). These measures allow the observer to determine an animal's welfare usually after the problem has occurred. Behavioural observations can also be used to help understand what an animal needs or prefers. This can be achieved by carrying out preference or choice tests, which will be discussed in the following section.

2.3.2 Assessing behavioural priorities: preference studies and animal welfare

When confining animals it is essential to cater for their needs, *i.e.* their physiological, physical, behavioural and psychological requirements, to prevent health and welfare problems. Such a 'needs' list would consider space, food and companionship with each factor potentially subdivided, for example, companionship will include the number of animals in the group and the group structure itself, *i.e.* sex ratios and ages. Such questions can be answered by having an understanding of the animal; knowledge of its natural behaviour, how it is adapted to cope with its environment and by knowing what it prefers under a particular set of conditions.

Preference tests offer a reliable method for understanding an animal's needs (Dawkins, 1980). In these tests the animal chooses one environment or resource over another. Such tests have been useful for designing environments that cater for an animal's requirements (see Beattie *et al.*, 1998; Van de Weerd *et al.*, 1998; Phillips *et*

al., 2000; Mills *et al.*, 2000). As welfare is concerned with understanding how an animal 'feels' about its environment (Petherick & Rutter, 1990), preference tests can also be used to reveal, to some degree, how positive or negative the animal 'feels' about this (Duncan & Kite, 1987). Preference tests offer an insight into how an animal is feeling because, as Fraser and Mathews (1997) state: 'an animal's preference is closely linked to its subjective experiences at the time of making the choice'. It is therefore possible to improve our understanding of an animal's needs through observation of its preferences. Dawkins (1980) adds that '...it may be possible to allow them to express at least some of their feelings, if not in words, then in actions'.

Preference tests are also known as choice tests or differential responsiveness tests (Martin and Bateson, 1995). Such tests offer the most direct method available for assessing welfare (Dawkins, 1980). In comparison, other measures such as health, abnormal behaviours and physiological responses are less direct. Essentially, preference tests can help us understand the animal's world (what it is thinking, how it is feeling and how it views a particular situation) (Dawkins, 1980).

It is an assumption of preference studies that the animal will select the resource that is most beneficial to its welfare at that time. This is suggestive of a relationship between what an animal selects and what it perceives to be most beneficial to it. Such a relationship is thought to exist because natural selection would have favoured such a connection (Dawkins, 1977). Broom and Johnson (1993) stated that animals are likely to select resources efficiently because natural selection promotes those behaviours that increase fitness. If an animal is not suited to its surroundings then it suffers and its survival is less good than an animal that suits its environment well (Broom, 1988).

Animals, therefore, have preferences for where they live as these environments increase their chances of survival. Webster (1995) also proposed that animals aim to avoid suffering and seek pleasure, and Tschanz (1987) suggested that all animals aim to satisfy their requirements.

Nevertheless, animals do not always choose what is best for them (Duncan, 1978). Broom (1981) demonstrated that rats had a strong preference for chocolate when offered a wide range of foods. Dawkins (1980) has also noted that rats and pigs choose a saccharine solution over a nutritious, well-balanced diet. It may therefore be our interpretation of the situation that is poor rather than the animal's choice; in the animal's world a sweet taste may indicate a rich food source (Dawkins, 1980). In order to cater for such misinterpretations, Dawkins (1990) suggests that preference tests should take place over a long period of time and other measures of animal welfare should be considered. Existing evidence suggests that animals do not make random decisions; instead, they make choices based on how they feel. These feelings are internally driven and this internal drive is termed the animal's motivation (Manning & Dawkins, 1992). An animal is motivated to modify its behaviour in such a way that its survival is increased and in doing so improves its welfare (Dawkins, 1980)

2.3.2.1 Motivation and behaviour

Motivation is controlled by the hypothalamic region of the brain (Dethier and Stellar, 1970). The hypothalamus has two behavioural functions – excitatory and inhibitory. The former is associated with the arousal of motivated behaviour whilst the latter is associated with the suppression of motivated behaviour (Dethier and Stellar, 1970).

Thus motivation is controlled by the brain and is responsible for initiating behaviour (Dellmeier, 1989).

As an animal does not respond to a given stimulus the same way each time it is encountered (Barnard, 1983) there must be a controlling factor, *i.e.* motivation, that dictates its response (Fig. 2.11). For example, an animal encountering a bucket of water will sometimes drink from it and at other times walk past it. An animal that is faced with several decisions will carry out the one which it is most motivated to do at that time (Fraser, 1974). This suggests that motivation controls which behaviours occur and when (Fraser & Broom, 1997) and directs an animal towards a goal (Dellmeier, 1989).

Fig. 2.11 The role of motivation in the stimulus-response relationship (Barnard, 1983)

Motivation is affected by three main factors (Fraser & Broom, 1997):

(i) Sensory stimuli: *i.e.* Information to the brain as perceived by the body's senses about the animal's environment, *e.g.* the smell of food may motivate the animal to feed. } External source

(ii) Internal inputs: *i.e.* Information to the brain from body monitors such as stretch receptors in the gut. In addition, these include information from the body clock indicating, for example, time to feed and sleep.

(iii) Hormonal state: For example, the release of sex hormones triggering mating behaviour.

} Internal sources

These factors will alter the behaviour of an animal and are collectively termed 'causal factors', *i.e.* 'interpretations of a wide variety of external changes and internal states of the body' (Fraser & Broom, 1997). Fraser and Broom (1997) also suggested that what motivates an animal to perform a particular behaviour is governed by the level of causal factors entering the decision making centre of the brain. As causal factors increase in intensity (or level) the motivation of an animal to behave in a way that reduces this drive is also increased (Fig. 2.12).

There are many causal factors being processed at any one time. It is those that are of a 'high priority' that motivate the animal to behave in a way that reduces the level of these causal factors (Fraser & Broom, 1997) (Fig. 2.13).

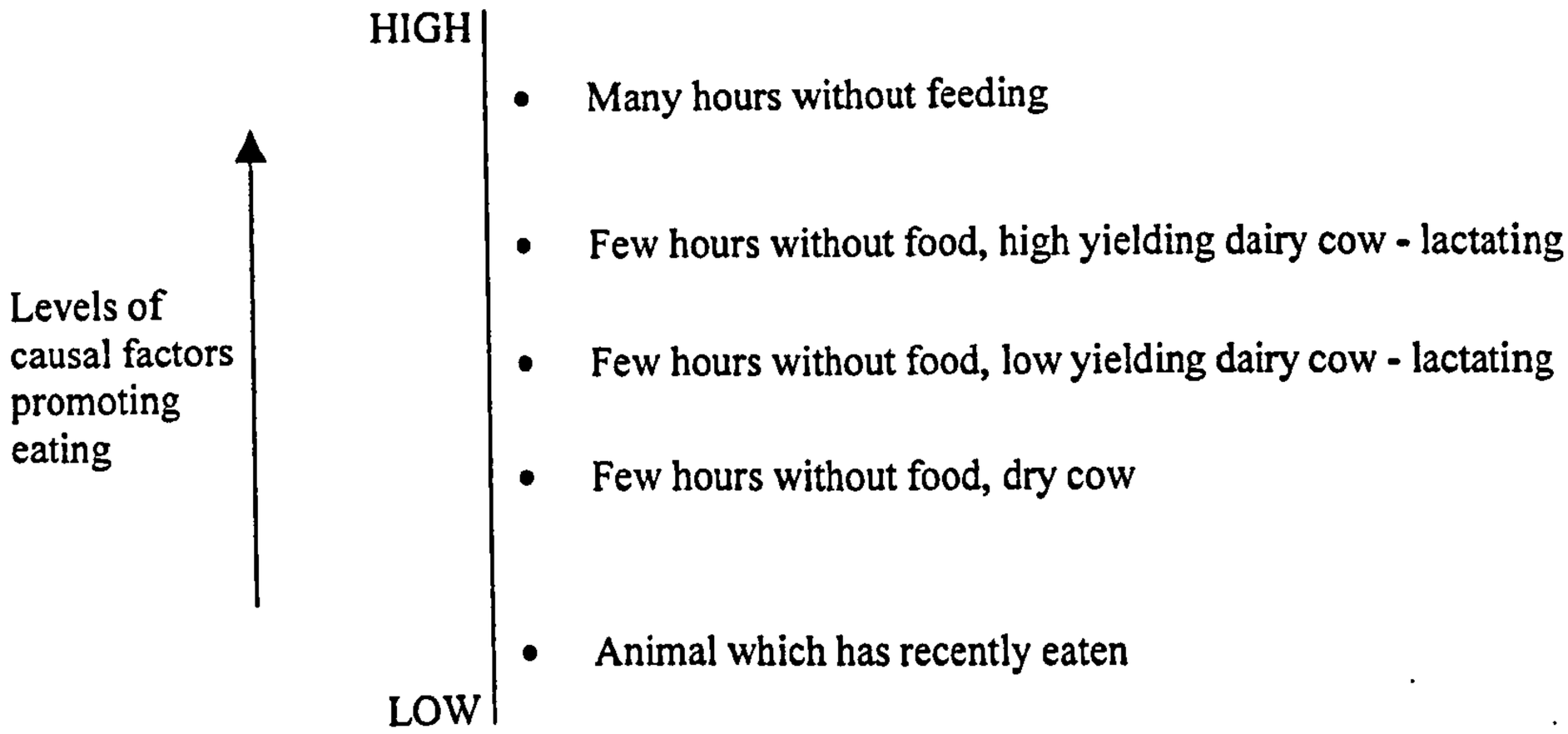


Fig. 2.12 As the levels of causal factors promoting eating in the dairy cow increase the motivation to feed also increases (Adapted from Fraser & Broom, 1997).

Causal factors ⟶ Decision making ⟶ Behavioural output

Fig. 2.13 The decision making process determines the behavioural expression of causal factors (Fraser & Broom, 1997)

An animal's motivational state, therefore, depends on the strength of a set of causal factors being experienced at any one time. The high yielding dairy cow can be used to demonstrate this. Due to a possible conflict between a need for food as well as rest, its state can be described and combined in a plot as suggested by Fraser and Broom (1997). In Fig 2.14 a cow who is at point *A* is more likely to eat and to behave in a manner that achieves this goal than if it were at *B*. Conversely, if it were at point *B* it would be more motivated to rest than if it were at *A*. The diagram illustrates the interactions between these states.

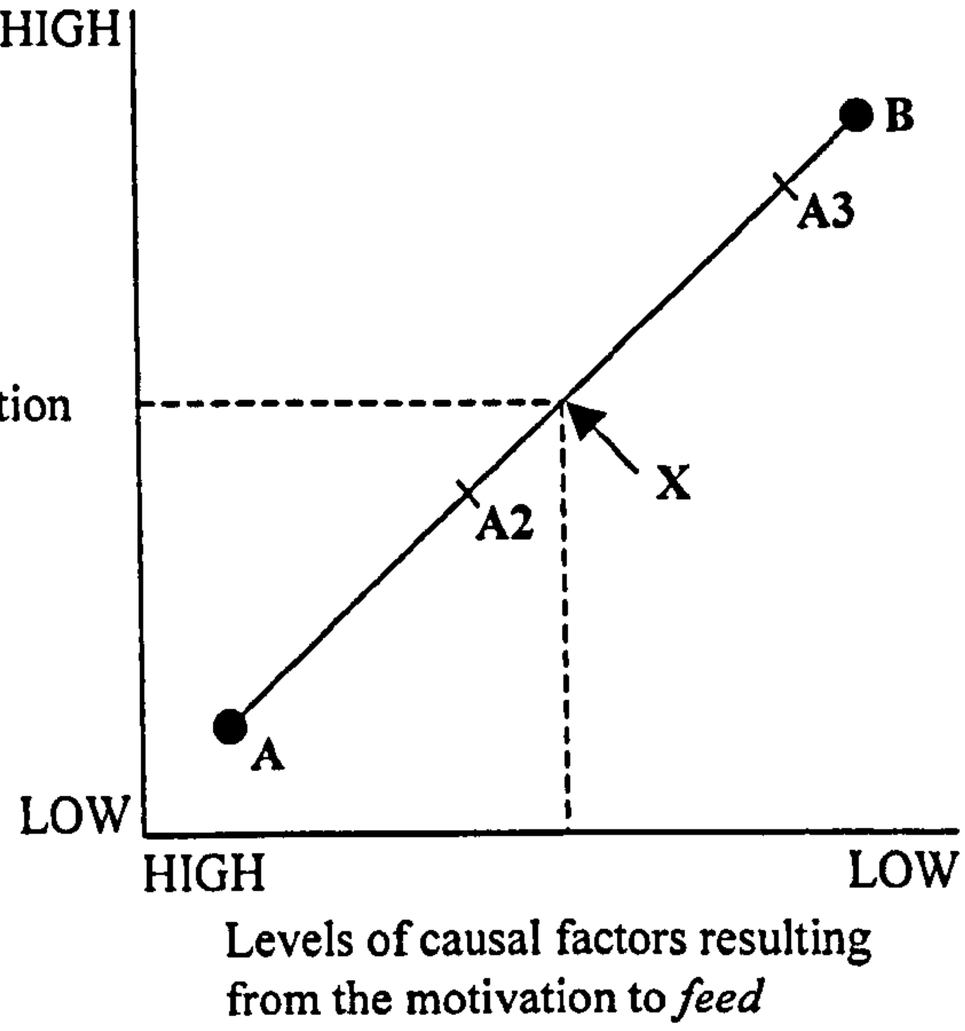


Fig. 2.14 Motivational state of the high yielding dairy cow

At certain times during the day, the dairy cow's motivational state moves from point *A* towards that of *B* (Arave & Walters, 1980), *i.e.* its motivation to rest increases. However, the high yielder may not exceed point *X* at the same time as the low yielder because it has not obtained enough nutrients to satisfy its elevated nutritional demand. The line from *A* to *A2* represents the change in state for the high yielding cow. Gradually, the high yielder will obtain enough nutrients and its motivation to rest will eventually exceed that to feed and therefore it moves through point *X* and onto *A3*. It is debatable whether the high yielder during peak lactation ever exceeds point *A3*, *i.e.* feels very little motivation to feed. The behaviour of the cow therefore depends on its state as represented in Fig. 2.14. A cow whose state is at *A3* is more highly motivated to rest, the performance of this behaviour would reduce intake, increase the level of motivation to feed and alter its state back towards *A2*. This would eventually bring its state below *X* at which time it may be sufficiently motivated to begin grazing again.

This information highlights the importance of considering motivational states when conducting preference studies. To obtain satisfactory results from such tests the animal must be in the desired motivational state as regards the aims of the experiment. For example, if bedding preferences are being researched then it is necessary for the animal to be in a motivational state that is promoting rest. If the same experiment were to take place during the day, when the animal was active, it may choose the bedding material that permits the active behaviours to take place most easily, rather than that which is most preferable to lie and rest on. In addition, motivational states are altered not only by internal but also by external sources. The experimental conditions may influence those decisions made by the animal. For this reason, results should be interpreted with regards to the experimental conditions from which they were gathered (Mills *et al.*, 2000).

Understanding motivational states can also help, to some degree, in determining how animals feel. This is because it can be assumed that an animal behaves in a certain manner at any particular time because it is motivated to do so in attempting to satisfy internal factors. From this view point, it is possible to use behavioural observations to determine subjective states. This can be demonstrated by the example of a cow grazing at midnight. As her motivation to rest should be exceeding that of grazing at this time, it is reasonable to suggest that the animal has a strong desire (*i.e.* is highly motivated) to feed, which exceeds the motivational desire to rest.

Motivation plays an important role in determining how an animal feels, how it behaves and therefore what it chooses. In this respect, preference tests provide a very useful tool in helping to understand the needs of an animal.

2.3.2.2 Simple methods for assessing preferences

It is possible to obtain some idea of what animals need and how they feel by conducting simple preference tests. Such tests involve providing the animal with two or more choices and seeing which one it chooses. This can give some idea of what an animal prefers, albeit dictated by its motivational state at that time. The preferences of animals can also be determined by simply observing what animals do in their 'natural' environment or an environment that permits a full range of normal behaviour patterns to be expressed (Broom, 1988). This is achieved by constructing an ethogram that details the behaviours displayed and the amount of time the animal spends in each activity (Manning & Dawkins, 1992). From this it is possible to select those activities the animal most prefers. A drawback of this method is that those behaviours that are only expressed occasionally, or for a relatively short period of time, may be considered as unimportant or least preferred, while this is not always the case. This is because the time spent displaying behaviour does not necessarily indicate its relative importance in terms of its necessity to be performed. This dilemma can be resolved by making the animal indicate how much the expression of a behavioural activity means to it. This can be achieved, in one way, by examining the animal's strength of preference to perform certain behaviours or gain access to different resources. Webster (1995) stated that this is a major problem with simple preference tests in that, on their own, they do not give any indication as to the animal's strength of motivation. It is therefore sometimes necessary to modify and extend the simple test in order to assess this.

2.3.2.3 Experimental techniques for assessing strength of preference

In preference tests it is important not only to ask the animal whether it likes something or not but also to indicate how much it values it (Dawkins, 1983a). A simple choice test does not indicate the relative importance of an animal's decision. It does not consider the value of its choice. Just because an animal selected option A over B, this does not necessarily mean that it will suffer if only provided with its least preferred option (Dawkins, 1983a). In agreement, Sainsbury (1986) suggested that a lack of preference for a particular environment or resource does not indicate that the animal will suffer if it is provided with this and not its most preferred one. Such arguments make it necessary to attach a 'cost' to a preference to determine its relative importance to the animal and in doing so assess its welfare value. This can be achieved by conducting experiments to test an animal's strength of motivation (*i.e.* how hard it is willing to work) to obtain a resource.

The more an animal wants or needs something the harder it is prepared to work in order to obtain it (Dethier & Stellar, 1970). This assumption forms the basis for assessing the strength of a preference. It enables the observer to attach relative values, or 'costs', to resources and discover how highly an animal values a particular commodity. A highly preferred option will contribute more to the animal's welfare than a less or weakly preferred one (Dawkins, 1990). To successfully measure the strength of motivation, an animal has to sacrifice something that is meaningful to it in order to obtain its desired choice. This may be food, time, effort or anything which conflicts with the animal's motivation to obtain its preferred option (Dawkins, 1983b). The strength of a preference can be determined by pitting a preference against

a known, valued resource, using limited time tests and aversion tests, by measuring work rate, and constructing demand curves (Dawkins, 1983a).

Pitting a preference against a known, valued resource involves the animal making a choice between the given resource and another resource which it is known to value. This could be, for example, food, or in the case of social animals, companionship. This test therefore sets up a direct motivational conflict. Rooijen (1980) carried out such a test when examining gilts' preferences for various floor types. When offered a choice of pens that did not vary in floor type, the pig would always choose the pen that was closest to another gilt – indicating a social preference. However, when one of the pens was covered with earth and the other left as concrete the pig would choose the earth flooring even when the other pen was nearer to another gilt. Thus, the earth flooring offset the social preference. This provides the observer with a relative preference value. It is known that pigs rate the company of their own kind highly, as they are social animals. Using this as a yardstick, it is reasonable to suggest that pigs have a relatively high preference for earth flooring. However, it is not possible to determine exactly how strong the preference for earth is, only to say that it is stronger than the preference for companionship. A more sensitive test might be to look at how much energy an animal is willing to expend to obtain its goal (Broom & Johnson, 1993).

In limited time tests the strength of preference is determined by observing which resource is chosen and for how long when time is limited. For example, Dawkins (1983b) designed an experiment to quantify the motivation to dust-bath in hens by providing them with the choice of either a cage containing food and water or one with

only litter. The hens had no access to either of these resources prior to the test. Dawkins (1983b) revealed that when the hens were given 8 h in the experiment they spent a majority of their time with the litter. However, when limited to 2 h the birds reduced their time spent with the litter to feed. The test works because the hen only has a short time to choose one or the other of the resources. As time is the limiting resource, the time spent feeding cannot be spent on dust-bathing and *vice versa* (Dawkins, 1983a). If time is severely restricted, so too is the potential for performing each of the activities. This will give some indication of the relative importance of a resource in comparison to the other. From this experiment, it could be concluded either that the hen's motivation to feed was higher than its motivation to dust-bath, or the hen had a high motivation to dust-bathe but only for a short time. This test does not tell us how the hen feels about not being able to dust-bathe. Maybe, then, the motivation to dust-bath should be measured independently. For example, the hens could be kept in a system whereby they have to work in some way to obtain the opportunity to dust-bathe. Hill *et al.* (1986) also conducted a limited time experiment on hens. They altered the time available to chicks to perform certain behaviours by varying the amount of light available to the chicks. Interestingly, they found that the chicks re-scheduled their behaviour. Instead of sacrificing the time spent in one activity for another, the chicks ate at a quicker rate giving them time to carry out other desired activities.

A requirement of limited time experiments is that the animals do not have access to any of the resources being offered in the experiment when not undertaking the test. Hursh (1988) demonstrated that food-deprived rats are less likely to work as hard for a food reward if they know they will receive food at the end of the experimental

period. In addition, Kirkden (2000) suggested that, for the same reasons, the animal should not be given access to a resource that satisfies the same motivation. An example of this is that a chicken might be less willing to work for sand to dust-bathe in if it is provided with woodshavings.

Aversion tests can be used to examine the strength of motivation in a number of ways. One method is to make the animal experience something unpleasant before it can reach its goal. The strength of motivation is determined by increasing the level of unpleasantness and observing at which point the animal avoids the stimulus and abandons its goal (Manning & Dawkins, 1992). For example, the animal may have to overcome an unpleasant experience, such as an electric shock, before it can obtain a food source. The intensity of this shock can be increased and the level at which the animal decides not to obtain food provides a measure of motivation. Cabanac (1985) made food-deprived rats leave a warm environment in order to feed in a cold one. Their feeding motivation was examined by seeing how prepared they were to overcome the cold to feed. The results revealed that the rate of feeding and the duration of the meals did not change but the number of meals, and therefore the amount of food eaten, increased.

An alternative method is to observe the animal's willingness to return to an unpleasant condition or avoid it. Such tests can be used to examine the aversiveness of certain handling procedures. Rushen and de Passille (1992) defined the aversiveness of a particular handling procedure as "the extent to which an animal seeks to avoid or escape from the treatment." This provides a tool by which different handling procedures can be compared. Rushen (1986) made sheep run down a race

and at the end they were subjected to a handling procedure used to restrain them during shearing. The sheep were either physically restrained or immobilised with an electric shock. The willingness of the sheep to run down the race to receive the same treatment was measured. The sheep were found to be more reluctant to return to the immobilisation method and so it was concluded that this was the more aversive procedure. A problem with these sorts of experiments is that the animal may be choosing the lesser of two evils (Duncan, 1992). The animal may show a preference for the less aversive procedure even though its welfare may be reduced by both. Gonyou (1991) adds that 'the [aversion] test cannot determine whether the choices are both pleasant, both aversive, or one of each.' In addition, such tests do not discriminate between different levels of pain (Rushen, 1986). Another method to measure feeding motivation is to contaminate an animal's food source with an increasing concentration of an unpleasant tasting chemical. The point at which the animal rejects its food provides some indication of its feeding motivation.

Measures of work rate require the animal to expend energy in order to achieve access to its most preferred choice. Duncan and Kite (1987) assessed hens' motivation to reach a nest box by measuring their willingness to overcome various obstacles such as a foot bath, a blast of air and a swinging door. The door was made increasingly more difficult to open by adding weights to it. The amount of energy the hen was willing to expend to open the door was considered to be indicative of its strength of motivation. Petherick and Rutter (1990) argued against the validity of this experiment because the door becomes increasingly difficult to open as it is pushed and the experiment is too long-winded because it involves slowly increasing the weight of the door over a number of trials. To overcome these problems they modified this approach and

developed a computer-controlled 'push-door'. This required the hen to push against the door with a certain force before it would open. The amount of 'work' the hen had to achieve was measured in terms of force x time (N sec^{-1}). They experimented with hens that were deprived of food for 12 or 43 hours. The hens were required to push against the door with a force of 13 N sec^{-1} before it would open and allow them to reach a food reward. They discovered that those birds deprived of food for 43 hours pushed through the door faster.

Operant tasks can also be used to measure work rate. Operant conditioning can take many forms but it essentially requires the animal to work in order to obtain a reward, gain access to a resource or change its environment. The animal may have to press a lever, button or plate, break a light beam, or operate a switch to receive a food reward. The amount of work an animal is prepared to do to receive the reward is indicative of its strength of motivation. Robinson (1998) suggested that the amount of work done is representative of the level of deprivation an animal receives. For example, Manning and Dawkins (1992) demonstrated that 'the rate at which water-deprived rats will bar-press... is reliably related to the length of time for which they have been deprived of water.' Operant tests have been very useful in gaining knowledge about the relative importance of certain resources. Operant experiments can be carried out in two ways:

1. Variable-ratio/interval reinforcement schedule. Here, the reward is given at irregular intervals, for example, one reward at a mean of one reward every 30 presses (ratio) or every 30 seconds (interval) (Manning & Dawkins, 1992). This means that the animal will not know at which bar-press, for example, or at what time it will receive the reward. The animal's response is relatively low but steady as it is unable

to predict when the reward will be available (Tarvis & Wade, 1995). Assuming the animal really wants the reward, it will press the bar continuously. Such a test produces reliable results to measure motivation, as the rate of work is very predictable (Tarvis & Wade, 1995).

2. Fixed-ratio/interval reinforcement schedule. This method delivers a reward after a fixed number of responses (ratio) or after a fixed length of time (interval) (Tarvis & Wade, 1995). For example, a reward may be delivered after every third bar-press. This method produces very high rates of responding (Tarvis & Wade, 1995).

In operant studies the animal can be positively or negatively reinforced. Positive reinforcers can be objects (*e.g.* food reward), physical changes (*e.g.* warmth) or the expression of behaviours (*e.g.* opportunity to dust-bathe in chickens) (Fraser & Broom, 1997). The animal may also perform to avoid a stimulus or terminate an unpleasant condition; a negative reinforcer. This can be a painful experience (electric shock), physical change (*e.g.* cold) or a frightening stimulus (*e.g.* gust of wind).

Kirkden (2000) pointed out that a problem with operant studies is that more or less any resource that is measured can turn out to be a necessity: "One problem may be that many operant tasks, such as panel pressing, are never hard enough that performing them constitutes a real effort for the animal." In addition, Kirkden (2000) adds that 'the experimental environment of an operant test typically provides the animal with little to do other than perform the operant task.' The task may therefore act as a possible enrichment activity providing the animal with something to do. However, a well-planned method could solve this problem. Broom and Johnson

(1993) stated that the demand for a resource may be considerably lower if the animal's motivation is stimulated to pursue other objectives at the same time. The motivation to dust-bathe in chickens, for example, could be examined by housing them in a standard open-range system and making them carry out an operant procedure in order to gain access to a dust-bathing area. This means they are within an environment where there are other things to do, however, if they feel the need to dust bathe the opportunity exists but with a cost attached. Such a method may be better able to quantify the necessity to perform such an activity.

Dawkins (1983b & 1990) combined economic concepts with ethological theory to produce a valuable method for estimating and comparing motivational strength from operant data. This is achieved by developing a 'demand curve' for a given resource and comparing it to a curve that is representative of an essential need - such as food. The curve considers both the 'price' of a resource and its 'demand'. The 'price' of a resource is determined by either how hard (effort) or for how long (time) an animal is willing to work to obtain it. The 'demand' is determined by the number of times the animal gains access to the resource. This method produces a graphical representation of the animal's demand for a resource and is therefore termed the 'demand curve' (Fig. 2.15). This curve describes the relationship between the demand for a resource and how hard the animal is willing to work for it.

Broom and Johnson (1993) stated that the shape of the demand curve will vary according to the size of the reward, the needs of the animal for the resource, and the effort or time required to carry out the operant procedure in relation to other demands upon the animal

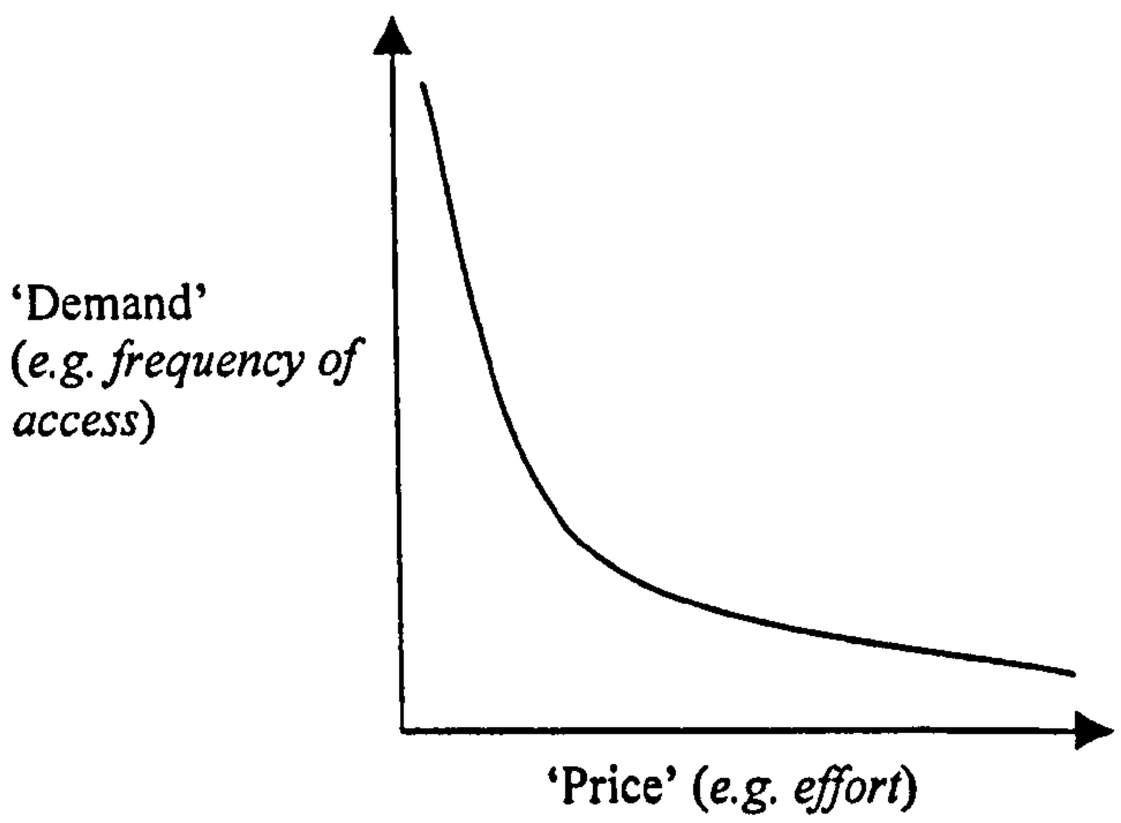


Fig. 2.15 A typical demand curve – as the 'price' increases the demand for that resource decreases (Dawkins, 1983b).

Lea (1978) stated that the fixed-ratio reward technique can be used to accurately estimate the value of a resource. If an animal continues to work at the same rate as work duration increases (*e.g.* it continues to press a lever at the same rate even though the number of presses increases) then its demand for that resource is termed 'inelastic,' *i.e.* this resource is a necessity. Alternatively, if its work rate declines as work duration increases its demand for that resource is termed 'elastic' and is therefore a luxury. The value of the demand curve is therefore termed the 'elasticity co-efficient' as it demonstrates the elasticity of a resource. A value of, or close to, zero (*i.e.* a flat line) indicates an 'inelastic' response whereas a value nearer to, or greater than 1.0 indicates an 'elastic' response (Fig 2.16). Dawkins (1990) stated that 'suffering is most likely to occur if animals are prevented from performing the activities or deprived of the commodities whose demand curves have the flattest slopes'.

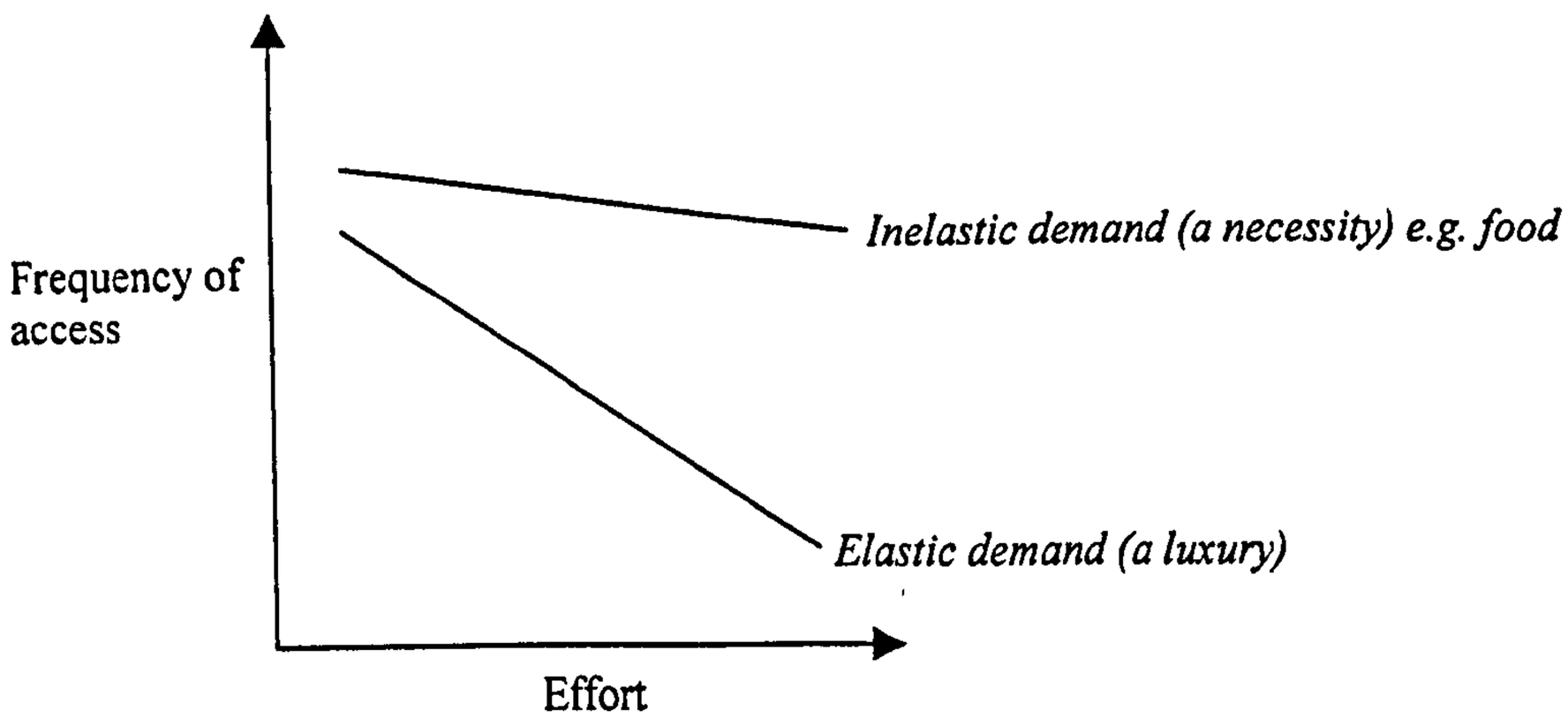


Fig. 2.16 Demand curves illustrating elastic and inelastic resources (Adapted from Broom & Johnson, 1993)

A problem with demand curves is to know at what point a luxury becomes a necessity. It is necessary to have an absolute criterion to which relative strengths of other preferences can be compared, because absolute preference cannot be determined (Gonyou, 1991). Dawkins (1983b) suggested that strength of preference can be compared against preference for an essential resource – such as food. If food deprivation is taken to mean that the animal is experiencing some degree of suffering, then other resources can be measured against this to assess their potential level of suffering on the animal (Dawkins, 1983a). Dawkins (1990) stated that those 'commodities with demand curves similar to that of food can be regarded as essential to welfare'. Motivation to obtain food is considered a useful yardstick as hunger is experienced by all sentient beings and Webster (1995) considered this "the most basic, primitive and unremitting of all motivational forces." However Dawkins (1983b) stated that food motivation might be too great a yardstick as it is unable to differentiate between lesser motivations.

2.3.2.4 Relative merits of preference tests

An animal's preference for a given resource can be influenced by many factors. It is important to take these factors into consideration when designing and interpreting information from preference tests.

An animal will make a decision based on how it is feeling at the time of testing. An animal's feelings can be altered by many factors, such as the time of day or year. Arey (1992) demonstrated that sows had a stronger preference for access to a food pen over one containing straw, except on the day prior to farrowing when the preference for both resources were equal (Table 2.7).

Table 2.7 Presses on a panel to obtain access to a straw or food pen (Arey, 1992)

Presses per reinforcer (fixed ratio)	Days before farrowing	Average number of presses for: Straw	Food
50 - 300	2	2.6	11.4
50 - 300	1	16.4	17.0

This demonstrates that animals will select what is best for them at the time, but not over a long-term period (Sainsbury, 1986). Dawkins (1990) stated that 'short-term choices made in response to an immediate need may not reflect the animal's long-term preferences.' In addition, an animal's initial choice may be due to novelty rather than long-term benefit (Broom & Johnson, 1993). Duncan (1978) also added that 'animals...cannot be expected to weigh up the long-term consequences of their decisions as would human beings, and to make rational choices accordingly.' This demonstrates the importance of carrying out long-term experiments and monitoring the welfare of animals over this period. To overcome this problem the animal can be

confined to its choice for a period of time before it is tested again (Hughes, 1975). If the animal knows that it has to spend a period of time in its chosen environment it may choose more carefully. This methodology may ensure that the animal is basing decisions on a longer-term basis.

Contrary to the effect of novelty described above, what an animal has previously experienced may also make a difference to what it prefers (Sainsbury, 1986; Fraser, 1985). Broom and Johnson (1993) state that 'preferred foods, companions, resting places and so on differ according to early and recent experiences.' This is important to bear in mind when generalising results to a wider population. Dawkins (1980) observed that battery-caged hens, when given the choice, initially preferred a similar cage to an outside run. However, this preference changed on the second and subsequent days. The hens had not experienced an outside environment before and so initially selected the more familiar environment. The new outside run, although being arguably better for their welfare, initially proved to be too unfamiliar.

Nicol (1986) indicates the importance of considering individual differences. When conducting housing experiments with hens she found that they sometimes spent time in the less preferred cages. She suggested that this may occur due to individual variability. The exact reason for this is not known. It may be that an animal is not showing a clear preference due to a negative emotional state (Broom & Johnson, 1993; Broom 1998). This may be a form of depression, such as apathy, unresponsiveness or learned helplessness. Broom (1988) states that preference test results may be falsified by using animals that have been living in difficult conditions for long periods of time. These animals may have adopted coping strategies and so

become particularly unresponsive. This makes it difficult for them to learn tasks and may affect the way the animal carries out the preference test.

Experiments have also revealed that some animals have a positional bias (Dawkins, 1980; Rooijen, 1980). The animal may have a natural preference for taking the option on the right as opposed to the one on the left, for example. Rooijen (1980) demonstrated that pigs have an individual side preference (side constancy). When conducting choice tests the pigs consistently preferred one side to the other. The pigs would even choose a barren environment over one containing a food reward. Rooijen suggests four possible reasons for this occurrence:

1. The pigs spent 5 minutes in the starting box prior to the test - this made them stressed and so reduced their motivation to feed.
2. The pigs developed a habit to select a particular side.
3. Animals often choose a known path into a known area in a flight situation.
4. The pigs were not motivated to feed and therefore associated the reward as being the termination of the experiment.

To minimise this side constancy Rooijen (1980) put the pigs through a 'correction procedure' and also minimised the amount of time they spent in the starting box (up to a maximum of 30 seconds). The 'correction procedure' was designed so that only the least preferred choice could be selected immediately after a trial. For example, if the pig chose the right-hand side on the first trial, it was immediately put through the test again but only had access to the left-hand side. Then, on a third trial the pigs were only offered a food reward in the previously least preferred area. In addition, they

were made to stay in the selected area for 5 minutes after each trial. This method of correction eliminated any side-constancy in the experiment. Interestingly, even when this side preference was eliminated it still made no difference to the results of the choice test. Although considered a problem, it can be advantageous to have this side constancy in determining the relative strength of a preference. Jones and Carmichael (1999) observed pairs of chickens housed in a wooden box from 0-5 days old. They found that the chicks favoured one side of the box to the other – indicating a side preference. On day 6 they introduced three enrichment objects to the least preferred side. They revealed that, after overcoming neophobia, there was an increased usage of the side containing the environmental enrichment objects. This. procedure demonstrated the relative effectiveness of the items as enrichment objects.

Different experimental designs, investigating the same question, can give contradictory results (Dawkins, 1990; Manning & Dawkins, 1992; Fraser & Mathews, 1997) as they can influence the decisions made by an animal. For example, an animal that has to lever press to gain access to a reward may not show the same degree of preference when it has to carry out an alternative activity. This problem has been encountered whilst investigating sows preferences for farrowing crate width. Baxter (1991) revealed that sows preferred a narrow crate whilst Phillips *et al.* (1992) concluded the opposite. The difference was due to experimental design. Baxter used four wooden partitions to create three variable-width, open-ended stalls in a large room. In contrast, Phillips *et al.* used three farrowing sites radiating from a central area. Fraser and Mathews (1997) suspect that the variation in results is due to one of two factors:

1. Because Baxter used open-ended stalls, it was the narrowest stall which provided the most visual enclosure; presumably making the pig feel more 'secure'; or

2. The preference for a wider stall in Phillips *et al.* design may have been so the pigs could turn around so as to walk out, instead of having to back-out. In Baxter's open-ended design the pigs were able to walk straight out.

Hughes (1975) also encountered similar problems when investigating hens' preferences for either a wire or litter flooring. In the first experiment hens were given constant access to both wire and litter flooring. The hens demonstrated no particular preference towards either floor type. Conversely, in the second experiment they were give a choice between separate cages, one with litter and the other with wire, and once they had chosen they were confined to the selected cage for several hours. In this situation there was a strong preference towards litter. In three experiments with water-deprived rats Miller (1956) cited in Manning and Dawkins (1992) observed that drinking motivation was not represented equally. To quantify motivation he examined the amount drunk, amount of unpalatable (bitter) water drunk and the rate of bar pressing to obtain water. It was revealed that the rats drank more water but did not increase bar-pressing activity up to 15 minutes after salt intake. However, 3 hours after salt intake the amount of water drank reduced but bar pressing and tolerance to unpalatable water increased. Manning and Dawkins (1992) suggest these differences can be explained because motivation covers a range of causal factors within an animal (hormones, neural activation, etc), which may not change in step.

Preference tests, especially operant procedures, require the animal to learn a procedure. The ability of an animal to learn will limit the complexity of the test that can be carried out. Problems may be encountered if the test itself is too confusing, complicated or frightening for the animal. The animal must have an understanding of the experiment and be able to associate the work being done with the reward being received (Webster, 1995). If the animal understands the task then it will make a more informed decision. Training the animal sufficiently, in part, can minimise such a problem. Training should aim to increase the animal's knowledge and experience of the test situation. Designing the task to be as close to the animal's natural behaviour patterns as possible will also aid learning (Dawkins, 1990). Requiring animals to perform unnatural tasks may not enable them to fully express their motivational level (Dawkins & Beardsley, 1986). Fraser and Mathews (1997) state that '[it is best] to base preference research on the types of choices that the species arguably evolved the capacity to make, and that the individual animals are accustomed to making in their normal lives.' In addition, preference tests should be set within the limits of the sensory and cognitive capacity of the animal (Fraser & Mathews, 1997). Jackson *et al.* (1999) demonstrated this problem by using two different methods to measure feeding motivation in sheep. In the first experiment the sheep had to push a panel with their noses to obtain a food reward. With this method only 25% of the sheep learned the task and motivation did not appear to increase with the increasing food deprivation (the sheep did not increasingly press the panel to receive a reward). However, in the second experiment, the sheep had to run down a race and enter the reward via a weighted door. In contrast, 70% of the sheep learned this task and motivation significantly increased with food deprivation. This was observed by more sheep going through the push-door, spending less time pushing the door, and being quicker to

reach the food than non-deprived sheep. The first experiment concluded that sheep were not highly motivated to feed after a period of food deprivation. However, it was the experimental design which failed to demonstrate this. The second experiment was successful because it used the animal's locomotion, which is more closely related to feeding behaviour than manipulation of a panel with the nose. Consequently, the task was learned more quickly and by more individuals.

Finally, Webster (1995) adds a word of caution and warns that preference tests should not be 'over-interpreted' or 'considered in isolation.' It is important to interpret choices from the animal's perspective and design experiments with respect to its world and with consideration to its behaviour (Dawkins, 1980).

2.4 Conclusions

The literature cited in this review suggests that the welfare of the high yielding dairy cow may be compromised by the metabolic demands of milk production (Wilson, 1978; Wildman *et al.*, 1982; Gearhart *et al.*, 1990; Harrison *et al.*, 1990; Veerkamp *et al.*, 1994; Gallo *et al.*, 1996; Rauw *et al.*, 1998). It is proposed that the grazing high yielding dairy cow probably suffers from the unpleasant emotional state of hunger, which is due to an inability to consume sufficient herbage to sustain a high level of milk production. Evidence for this has been concluded both from an assessment of the animal's physical condition and observations of the time spent in food-directed behaviours. The following observations, made in comparison to lower yielding dairy cows, provide evidence for this proposal: The high yielding cow undergoes a longer and higher loss of body condition during peak lactation (Gallo *et al.*, 1996) resulting

in emaciation; the high yielding cow takes the opportunity to graze at abnormal periods during the night, which has been termed the 'midnight snack' (Phillips, 1998); and, high yielding cows extend their grazing period, which may be achieved by grazing earlier in the morning (Phillips & Rind, 2002) and later at night (Phillips & Denne, 1988). In addition, Phillips (2001) stated that the high yielder may increase feed intake by increasing biting rate and dry matter intake per bite. The high yielder may therefore have to employ a range of behavioural coping strategies in response to the metabolic demands of milk production. Although this allows her to cope in this respect, other important behaviours such as lying down to rest (Phillips, 1993) may possibly be neglected.

It is therefore the aim of this project to examine the behavioural needs of the high yielding dairy cow. Initially, this will be achieved by employing a combination of grazing behaviour observations and an operant conditioning test. Grazing observations will identify those strategies adopted to cope with obtaining sufficient nutrients to sustain high levels of milk production. The operant test will be employed to determine the relative motivational strength to feed between high and low yielding cows.

The outcomes of these enquiries will benefit not only the animals themselves but also the dairy farmer, as consideration of the high yielders nutritional requirements may reduce the incidence of health problems that can result in a reduction in milk yield and, in extreme cases, culling.

3a. An investigation to establish the social interactions existing between dairy cows kept indoors on a straw-yard based system

3.1a Introduction

Concern has been expressed regarding the validity of using individual dairy cows as replicates in the statistical analysis of their behaviour at pasture (Rook & Huckle, 1995). Cows are social animals and graze in groups (Phillips, 1993) thus the possibility exists that the behaviour of some individuals within a herd could be dictated by other, possibly more dominant, cows. For example, Rook and Huckle (1995) believe that the grazing behaviour of dairy cows may be socially facilitated, with the commencement and duration of this period being dependent on a few individuals within the group. If shown, this could have implications for the validation of certain statistical techniques such as analysis of variance (ANOVA). The use of ANOVA requires that replicates are independent of each other (Snedecor & Cochran, 1978) and such an occurrence would invalidate the use of this method as a means of statistical analysis. This allegation has primarily arisen from an observation of the synchronous behaviour of dairy cows whilst grazing (Rook & Huckle, 1995). However, the existence of further evidence to support this is absent. As the true extent and effect of allelomimetic behaviour on the interdependence of cattle behaviour has yet to be fully understood, researchers must ensure steps are taken to cater for such effects. Therefore, in order to justify the use of ANOVA techniques in investigating the grazing behaviour of dairy cows it is necessary to consider the likelihood of such an occurrence. One method, suggested by Phillips (1998 & 2000a), was to identify and select those cows that have not been regularly observed together and use these individuals for further studies. This can be achieved within a group of cows by recording their positions on several occasions when the cows are close to each other.

Employment of such a technique would suggest that those cows selected as not regularly together could be considered as behaving independently, which would then justify and validate the use of ANOVA techniques.

3.2a Materials and Methods

3.2.1a Animals

The experiment, which was conducted over a six week period between 16th April and 28th May 2001, used cows from the Holstein-Friesian dairy herd of Moulton College, Northampton. The management system employed dictated that the herd of 200 dairy cows was divided into three lactating groups according to stage of lactation: High yielding (cows at peak lactation), mid yielding, and low yielding (cows nearing end of lactation). The high yielding group was selected for study, consisting of 60 dairy cows, as they contained the widest range of individual milk yields at the time (25.0 – 45.6 kg d^{-1}), which was desirable for a following experiment. This group was also expected to stay constant for the duration of the experimental period, with no cows expected to either leave or join, thus ensuring that the relationships within the group remained stable. The members of this group had been together since early March and individuals were therefore familiar with each other and had the opportunity to establish a stable hierarchy. The herd had not previously been used for experimental purposes.

The cows were observed during periods when they were able to choose whom they came into contact with. The positions of all cattle were recorded when they were (a) lying down, (b) at the feeding barrier and (c) entering the milking parlour, to

determine which cows came into close contact and could not be considered independent.

3.2.2a Conditions

For the duration of the observations, the cows were housed together in a large straw-bedded shed (70 x 15 m). During periods of darkness, artificial lighting was provided at either end of the shed. All cows were given *ad libitum* access to drinking water and a commercial total mixed ration [grass silage (37.9%), maize silage (32.9%), concentrate pellets (Moulton Balancer, Heygates Ltd, Northampton, UK) (14.0%), brewer's grains (13.2%), lucerne pellets (1.7%), and Megalac (Volec UK, Royston, UK) (0.3%)]. Cows were milked twice daily through an Alfa Laval 16:16 herringbone parlour, between 05.15 and 06.30 h, and 17.15 and 18.30 h, and were each provided with 2 kg of concentrate (Grassmaster 18, Heygates Ltd, Northampton, UK) per milking. Feed was delivered once per day during morning milking. All cows were in good health at the start of the study and in particular had no obvious signs of mastitis or clinical lameness. Freeze brand/ear tag numbers were used to identify individuals.

3.2.3a Lying positions

Cows were observed on 12 separate occasions between 30th April and 25th May 2001. Data collection took place from c. 20.15 - 21.00 h when the majority of the cows had finished feeding and started to lie down. A previous pilot study revealed this to be the most appropriate period as the majority of cows were lying during this time. All those cows lying within a 1 m radius of the observed cow's trunk or head (not including legs and tail) were recorded and regarded as an animal interacting with that cow. A list of interacting animals was compiled for each member of the group. A 1 m

threshold was considered sufficient as ample space allowance enabled cows to lie at a greater distance if required.

3.2.4a Feeding positions

The group was observed on nine separate occasions between 30th April and 25th May 2001. Data were collected between 18.30 and 19.00 h post-milking when the last cows of this group had left the milking parlour and lined up at the feeding barrier. A previous pilot study revealed this to be the most appropriate period as the majority of cows would choose to feed after being milked. The two nearest neighbours (within 1 m) either side of the observed cow were recorded and regarded as her feeding partners. This was carried out for each member of the group. Where the feeding barrier was disrupted by a gate, those cows feeding nearest to this gate only had one feeding partner, as did those feeding at either end of the barrier.

3.2.5a Order of entry into the milking parlour

Cows were milked twice daily, commencing at c. 05.15 and 17.15 h. Observations took place on three separate occasions at afternoon milking between 16th and 28th May 2001. As the order of entry is generally consistent over a period of time (Phillips, 2001) only three observations were considered necessary. The nearest neighbour either side of each cow in the group was recorded. Cows at the end of a milking row had only one recorded neighbour.

3.2.6a Dominance hierarchy

An additional method of identifying animals that interact is to establish a dominance hierarchy. This could also be used to select those individuals that do not interact with

each other. The experimenter attempted to compile a hierarchy for the group but this was not possible as the existence of those behaviours used to maintain a hierarchy, such as butting, visual threats, bunting and grooming others were scarce. This was thought to be due to the small group size and the long period in which the cows had been together. These factors aid recognition amongst individuals and promote group stability.

3.3a Statistical Analyses

For each cow the number of times it had been seen with another group member was determined for the three activities. All data for each measure were then combined to produce a table detailing the number of times an observed cow had been seen with another group member out of the 24 possible occasions.

For the feeding data, a predictive feeding association model (Webb, 2002) was used to determine the number of pairs of cows which would be expected to occur together on two or more occasions if the cows were mixing at random. The simulation worked with 60 cows, numbered one to 60, and randomly ordered these within a column as a list. Nine such lists were created to represent the nine observation sessions that took place. At the 15^{th} cow, on each list, a 'gap' was put into the data to represent the gate in the feeding barrier. This ensured that an extra pair was not counted, as the 15^{th} and 16^{th} cows were never paired and thus not recorded as feeding partners. Cow 15 was selected at random for this procedure. The first 26 cows of each list were used to create a distance matrix (Table 3.2a), which shows the number of times each cow was observed with every other cow over the nine observation sessions. Only the first 26

cows were used, as this was the mean number of cows observed per session. The matrix can be used to determine the total number of pairs occurring together twice, three times and so on. This procedure was carried out 500 times to obtain a confidence range of the expected number of pairs of cows that would occur together if feeding partner selection was random. The highest and lowest 2.5% of these values were rejected as possible outliers, providing a 95% confidence band.

The Kendall's Coefficient of Concordance was used to determine whether the order of entry into the parlour was consistent between observations. This was performed using the procedure described by Siegel and Castellan (1988). The test expresses the degree of association that exists between more than two sets of rankings of individuals by computing the coefficient value (W), which ranges between zero (no association between rankings), and +1 (complete association between rankings) (Siegel & Castellan, 1988). The probability value was determined by using a chi-square table at a 95% confidence interval.

A cluster analysis was performed on the combined data using the 13th release of the Minitab statistical programme (Minitab, 2000). This uses a distance matrix to produce a dendrogram, which clusters pairs of animals based on their similarity of interactions with other members of the group. This was used to determine which cows had been observed to interact with the same cows, and is a recognised technique to determine associations between individuals within a large group (Martin & Bateson, 1995).

3.4a Results

3.4.1a Lying positions

Over the 12 observations, only seven pairs of cows were observed together (within 1 m) on two occasions, three of which shared one common cow. No cow was observed with any other individual more than twice.

3.4.2a Feeding positions

Over the nine observations, only ten pairs of cows were observed together on two occasions, two of which shared one common cow. No cow was observed with any other individual more than twice. The predictive feeding association model revealed that, with random associations, between five and 16 pairs would be observed together twice, 95% of the time. Also, it predicted that between zero and two pairs would occur together three times, and no cow would be expected to occur with any other individual more than three times.

3.4.3a Order of entry into the milking parlour

The Kendall's Coefficient of Concordance demonstrated that there was a high level of association between individuals across the three observations (coefficient value: 0.709, P=0.01). This demonstrates that the cows entered the parlour in a similar order for each observation, *i.e.* they were consistent in their interactions between individuals.

Over the three observations, six pairs of cows were observed together on two occasions when they entered the parlour, two of which shared a common cow. No individual was observed with a particular cow on all three occasions. Thus, although

the order is similar between observations, there is variation in the exact order within each row.

3.4.4a Combined data

The data collected were combined to provide the total number of times an observed cow had been seen with another group member across all observations. This was used to determine whether the total frequency of pairings observed were different from those that would be expected to occur at random. Forty seven (78% of the group) cows were observed with at least one other cow on two occasions (Table 3.1a). Nine cows (15%) were never observed with the same cow more than once.

Table 3.1a The number of cows each observed cow interacted with on two occasions

Number of observed cows	Number of cows each observed cow interacted with twice
11	1
17	2
14	3
2	4
1	5
1	6
1	8

Table 3.1a shows that it is most common for a cow to have interacted with two others. Twenty one cows (35% of the herd), 17 of which were from the 47 cows above, were observed with a single cow on three occasions. Three of these cows were observed with two cows on three occasions. No cow was observed with any other cow more than three times.

In summary, 15% of the cows were observed with at least one other cow only once, 50% were observed with at least one other cow only twice, and 35% were observed with at least one other cow three times.

3.4.5a Cluster analysis

A dendrogram was produced from a distance matrix, which details the number of times each cow was observed with every other cow over the 24 recorded occasions (Table 3.2a) (see appendix 1 for complete distance matrix).

Table 3.2a A distance matrix showing the number of times a cow was observed with every other group member (numbers shown are fabricated)

Cow No.	1	2	3	4
1	0	1	1	2
2	1	0	1	2
3	1	1	0	1
4	2	2	1	0

The analysis works by comparing all the rows with each other and then grouping (or clustering) them according to their similarity. It starts by clustering those rows that are the most similar first. If two cows, *i.e.* two rows, have a similarity level of 100% then they will have been observed to have the same frequency of interactions with the same cows. However, this result does not suggest that these two cows interact with each other, as they could have been observed with the same cows but on different occasions. A problem with this row by row comparison is that no two cows will ever have a similarity level of 100%. This is because a cow does not interact with itself and will have a zero value in this box (Table 3.2a). This zero value will come at a

different position for each cow, for example, in Table 3.2a, cows one and two interact with the same cows the same number of times but their rows differ by the positioning of their zero values. To overcome this it was necessary to determine the maximum similarity level (%) that could be achieved if two cows interacted with the same cows the same number of times. This was examined by artificially adding another cow (number 61) to the distance matrix, which had the same frequency of interactions with the same individuals as another cow within the group (cow 60 was selected for this purpose, see Fig. 3.1a – sixth cow from left). This procedure produced a similarity level of 94.3%. This figure can be taken as a benchmark, representing an artificial 100% level of similarity. Thus, any two cows achieving a similarity level of 94.3% could be considered as having the same frequency of interactions with the same individuals.

The dendrogram (Fig. 3.1a) shows that no two cows within the group were observed to interact with exactly the same cows. Cows 16 and 55 achieved a similarity level of 93.1% (Table 3.3a) (see appendix 2 for complete dendrogram). The next level of similarity was at 89.4% between cows 16 and 37.

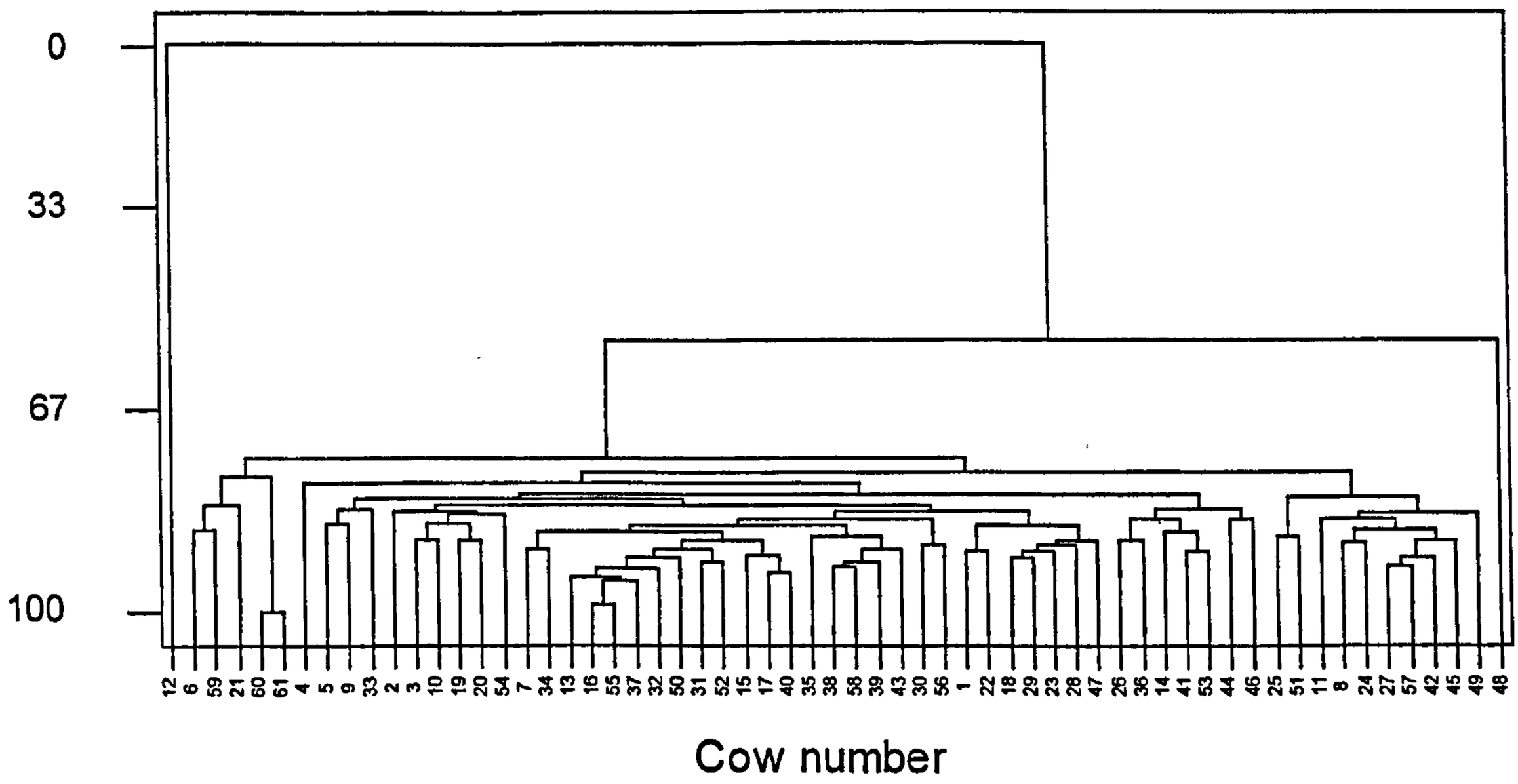


Fig. 3.1a A Dendrogram showing cows clustered according to their level of similarity (%) in their interactions with other individuals. Note, cow 61 is a 'dummy variable' programmed to be identical to cow 60, and therefore their similarity is 100% - the Y-axis has been adjusted to indicate this (see text)

Table 3.3a The similarity levels (%) for the first ten pairs of cows in their interactions with other cows

Cows clustered	Similarity level (%)
60, 61	94.3 (artificial 100%)
16, 55	93.1
16, 37	89.4
13, 16	88.7
17, 40	89.0
13, 32	87.3
38, 58	86.7
27, 57	86.7
31, 52	86.1
38, 39	86.1

The dendrogram can be 'cut' at a similarity level where those interactions taking place can be considered relevant to the experiment, *i.e.* to determine at what level two cows are to be considered similar enough in their frequency of interactions with the same cows. This decision has to be largely subjective (Minitab, 2000). Minitab (2000) suggests that this can be achieved by cutting the dendrogram where the similarity levels change abruptly, if this suits the data. Alternatively, it may also be possible to determine this point by plotting the distribution of the similarity levels (%) and cutting the dendrogram where they occur most frequently. It should be noted that the dendrogram does not provide any indication of interdependence taking place, as it does not show those cows that directly interact with each other. Nevertheless, it does show which cows interact with similar cows and to what extent. This may provide some indication of grouping effects within the herd, but requires further investigation.

The nature of the data collected for lying made it difficult, when combined with the data for both feeding and order of entry, to model and calculate the likelihood of two cows being observed together on two or more occasions (Webb, 2002). This was due to the highly variable number of cows an observed cow could have been lying with on any one recording (between zero and five other herd members). With the other measures, the observed cow could have only been with up to two other cows on any occasion, facilitating statistical analysis of this data. For this reason, probability values are not presented for the combined data. Nevertheless, it is possible to make a judgement about the data, which can help to understand the significance of those interactions. For reasons which will be explained later (see discussion), the data for order of entry into the milking parlour was discounted as it was believed not to be fully suited to the exploration of interdependence amongst dairy cows. When the data

for all three measures were combined, it revealed that no pair was observed together more than three times. If the results for each measure are examined separately, with this taken into account, it is possible to suggest that the occurrence of those associations observed amongst herd members is sufficiently small that they are likely to be occurring by chance. This is because, in each of the three measures recorded, no pair was observed together more than twice. This indicates that if a pair had been observed together twice in any one measure, then they could only be observed together again in only one of the other measures. For example, seven pairs of cows were observed lying together on two occasions out of 12. Therefore, it is only possible for these pairs to have been observed together again on one other occasion, *i.e.* either whilst feeding (once out of nine recordings), or, on entering the milking parlour. Similarly, nine pairs of cows were observed together twice when feeding, and they also could have only been observed together on one other occasion, *i.e.* whilst lying, or, entering the parlour. From this viewpoint, it is reasonable to suggest that if these pairings were non-random, then we would expect them to have occurred more frequently in any single or combined measure.

3.5a Discussion

It is expected that the results obtained in this study are largely herd specific, and should be considered with this in mind. The Moulton College cows could be considered as a 'non-research' herd and for this reason may be more likely to show associations if they exist, as experimental herds generally undergo frequent changes between groups. With the adoption of this methodology, 85% of the cows within the group were observed with another cow on at least two occasions. Forty one percent

(*i.e.* 21 cows) of these cows were observed with another cow on three occasions. No cow was observed with another more than three times out of the 24 recorded occasions. A judgement of the data suggests that those pairings observed were likely to have occurred at random and thus cannot be used to argue in favour of genuine associations. On such grounds, this research cannot propose that these cows were behaving interdependently, *i.e.* that the behaviour of either cow was being influenced by the presence of the other, as an association would be an expected prerequisite for this to occur. Considering these findings, this study does not support the contention of Rook and Huckle (1995) that cows are interdependent. Their suggestion of the occurrence of interdependence was based on the observation of dairy cows synchronised behaviour during grazing. This is not sufficient to infer that the behaviour of cows is interdependent. Phillips (1998) suggested that the synchronisation of behaviour is predominantly influenced by environmental factors, such as photoperiod, weather and management practices rather than allelomimicry. Evidence to support this came from Phillips and Denne (1988) who noted that variation in grazing times exists between cows grazing together. They found that there was a between-cow coefficient of variation for grazing time over 24 h of 24%. Also they observed that the total bite number had a higher variation than grazing time, demonstrating that other aspects of grazing behaviour are also individually dependent. Bao *et al.* (1992) also observed that dominant cows tend to graze for longer than subordinates, which is dismissive of the occurrence of interdependence. Similarly, although dairy cows have been observed to synchronise their lying (Atkeson *et al.*, 1942; Schmisseur *et al.*, 1966; O'Connell *et al.*, 1987), Wierenga and Hopster (1990) propose that this synchronisation was more related to the commencement of lying rather than the duration of total lying time, which presumably remains individually

dependent. This is probably also true for grazing behaviour, where initiation is synchronised but not the termination of a bout. Lying synchrony has also been shown to vary depending on the housing system the cows occupy (Wierenga & Hopster, 1990).

It is possible that the methodology adopted was not fully suited to detecting the occurrence of interdependence. This may have been the case when observing order of entry into the milking parlour. The cows entered the parlour in a similar order for each observation, suggesting that they were consistent in their interactions between individuals. However, it is not possible to determine the nature of these interactions. For example, this does not imply that the order was determined by each cow's preference to be in close contact with another individual, or, that there was some order being manipulated by certain cows within the group: such as is found in a dominance hierarchy. In fact, there is only a weak relationship between dominance and milking order (Phillips, 1993). Other factors, operating at this time, are more likely to be accountable for this effect. Phillips (1993) stated that milking order is much more likely to be determined by cows' individual need to be milked, *i.e.* high yielding cows have a greater need to be milked and this encourages them to enter the parlour first. This factor makes milking order dynamic over time as some cows will be at peak lactation, therefore increasing their need to be milked, and others will be reaching or have passed this stage. This increased motivation to be milked is thought to derive from a build up of pressure within the udder (Phillips, 1993). High yielding cows are also thought to be attracted into the parlour by the concentrate provided due to an increased appetite at certain stages of lactation (Phillips, 1993). As several cows within the herd are likely to reach peak lactation simultaneously, there must be other

systems present to determine the order of entry at this level. This is where cow status may take precedence and dictate who enters the parlour first. It would seem that order of entry into the milking parlour, although convenient to measure, is not a good determinant of the occurrence of associations amongst group members with regards to this methodology. Nevertheless, it could be used to support the occurrence of independent behaviour. If cows were acting interdependently, they would be expected to enter the parlour with the same group members over time, irrespective of milk yield effects. This would only be the case if sociality had priority over the need to be milked. Further research is required to validate this assumption. In this study, feeding positions may also have been affected by those factors determining order of entry into the parlour, as cows were monitored immediately post-milking. Cows departed from the parlour in the same order as they entered and may have followed one another to the feed trough. This effect was not examined during the study. However, the results from the predictive feeding association model indicate that that the cows were mixing at random during this activity. The simulation calculated that between five and 16 pairs would occur together twice whilst feeding. As only 10 pairs were observed, this fits within the range of random association prediction. No pairs were observed together more than twice, which was also expected if cows had no partner preference. Observed pairings below or above this range would indicate that either the cows were deliberately avoiding each other, or that they had preferred partners, respectively.

It is likely that not all the possible parameters of interactions between herd members were monitored in this experiment (Phillips, 2001). However, Phillips (2001) pointed out that no methodology is perfect and that this one can be regarded as highly

preferable to the use of isolated or small groups of cows as it enables those group members to remain in a normal herd structure.

In conclusion, the results from this study cannot be interpreted to support interdependence of feeding, lying or milking behaviour between cows. Further measures are required to test this hypothesis. The methodology used here is primarily useful to determine the partnerships within a group and thus offers a useful starting point for the study of interdependence. For, before interdependence can be studied, it is necessary to determine whether cows within a group are associated. As partnerships may be superficial and change with time, as is seen with order of entry into the parlour during the course of a lactation, lengthy studies are required to confirm whether observed associations are genuine. It is the impact of the associations that then needs to be examined to determine whether the behaviour of those individuals is affected. This could be achieved in two ways. The first is to examine the behaviour of cows that have been observed to form an association. Then, on removal of a member of the partnership, to see whether their behaviour is significantly altered. Alternatively, the behaviour of identified associates within a herd could be compared with other non-associated herd members to determine whether the associated cows were more similar in their behavioural patterns, *i.e.* to determine whether the behaviour of associated cows is more similar than that of cows which are not associated. It is maintained that behaviours such as lying and feeding in cows may be considered independent until there is strong evidence to suggest otherwise.

3b. Differences in the behaviour of high and low yielding dairy cows Selected by genetic merit

3.1b Introduction

The impact of a high milk yield on the welfare of the grazing dairy cow is unclear (FAWC, 1997). Phillips (2001) has suggested that the grazing high yielding cow suffers from the unpleasant emotional state of hunger due to an inability to consume sufficient herbage to sustain a high milk yield. In an attempt to overcome this, the high yielding cow may have to modify her behaviour to increase herbage intake. For example, Phillips and Denne (1988) observed that high yielding dairy cows extended their grazing period in order to increase herbage consumption, which may have been achieved by grazing earlier in the morning and continuing later into the evening in comparison to their lower yielding counterparts. Phillips (1998) also noted that the high yielder may also graze during the night. This is particularly unusual behaviour considering the cow's innate fear of predators (Phillips, 1998) and the difficulties associated with herbage selection at low luminance levels (Phillips & Hecheimi, 1989). The employment of such coping strategies may also compromise the cow's welfare in other areas. For example, an extended grazing period may result in the cow having to neglect the expression of other important behaviours such as lying down to rest (Phillips, 1993). As Metz (1985) has revealed that cattle have a strong motivation to lie down, an increased grazing period may demonstrate the severity of hunger being experienced by the cow and thus imply a significant impact on her welfare.

The following experiment was designed to investigate whether, and in what ways, the grazing, high yielding dairy cow has to modify her behaviour in order to cope with the increased nutritional demands of milk production. From this it may be possible to

determine whether such strategies, if present, result in her having to neglect the expression of other high priority behaviours. This was investigated by comparing certain behavioural parameters of high and low yielding cows. Ethograms for each cow were constructed to determine the time spent in bouts of different behaviours over a 48 h period. Other aspects of ruminating and grazing behaviour were also recorded. For ruminating behaviour, this comprised of the interbolus interval and the rate of chewing, and for grazing this included the rate of biting, and the rate and extent of sideways head movement. Bout lengths and rates for the different activities were calculated from this data to determine the strategy of high and low yielding cows to make best use of their time.

3.2b Materials and Methods

3.2.1b Animals

The experiment, which was conducted between 21st June and 3rd July 2001, used cows from the Holstein-Friesian dairy herd of Moulton College, Northampton. A group of 60 lactating dairy cows was initially observed over a six week period (16th April to 28th May). This was to record the positions of all cattle when they were lying down, feeding and entering the milking parlour, to determine which cows associated with each other and may not be considered independent (see Chapter 3a). As a precautionary measure, to prevent possible problems associated with interdependence, 40 cows were selected that were not observed to have associated with each other more than twice out of a possible 24 occasions. These cows were divided into two groups of 20 high yielding and 20 low yielding cows, with mean milk yields of 40.6 kg d^{-1} (s.e. 0.51) and 31.0 kg d^{-1} (s.e. 0.75) respectively ($P<0.01$) based on National Milk Records (NMR) taken prior to the commencement of the study (16th May) (Table

3.1b). Further milk recordings were taken eight days prior to, on the first day of and nine days after the study (Table 3.2b).

Table 3.1b Characteristics (cow no., body condition score (BCS), lactation number, days in milk, and milk yield) of the experimental animals selected for high and low milk yields

Low yield					High yield				
Cow No.	Mean BCS	Lactation No.	Days in milk	*Milk yield (kg)	Cow No.	Mean BCS	Lactation No.	Days in milk	*Milk yield (kg)
42	2.83	2	243	25.0	95	2.92	3	280	37.0
14	3.50	3	220	26.4	4	2.75	3	214	37.2
579	3.25	5	274	26.4	129	3.00	3	208	38.0
72	3.58	2	305	27.6	300	3.08	4	178	38.0
5	2.92	5	202	27.8	230	3.08	6	250	38.2
551	2.92	4	243	28.0	567	2.67	3	206	38.6
21	3.08	3	285	28.6	180	3.25	3	251	39.4
236	3.50	4	288	29.8	544	3.00	4	215	39.8
540	3.00	6	250	31.0	572	2.75	3	247	40.2
101	3.17	9	169	31.2	549	2.83	3	219	40.4
19	3.00	2	204	31.6	577	2.75	5	242	40.6
537	3.00	6	192	32.0	552	3.00	2	303	41.0
563	3.33	4	216	33.2	82	2.83	3	162	42.0
185	3.33	4	243	33.4	106	2.58	3	133	42.0
112	2.92	2	164	33.6	89	2.50	3	143	42.2
74	2.67	3	305	34.0	36	2.92	3	158	43.0
37	2.67	3	209	34.6	559	2.83	4	204	43.0
509	3.08	5	267	34.8	553	2.92	4	133	43.4
588	2.83	5	230	35.4	210	2.58	8	164	43.8
138	3.25	3	247	35.6	152	3.00	6	167	44.2
Mean	**3.09**	**4**	**237.8**	**31.0**		**2.86**	**3.8**	**203.9**	**40.6**

*Milk yields recorded on 16th May

Table 3.2b Mean milk yield data taken eight days prior to, on the first day of, and nine days post study for the experimental animals selected for high and low milk yields

Date of recording	Yield group	Mean milk yield (kg)	SED ±	Milk yield range (kg)	P-value
13th June (-8d)	Low	30.6	1.27	18.8 – 36.0	<0.01
	High	40.0		34.6 – 47.4	
21st June (first day of study)	Low	29.6	1.44	17.4 – 37.0	<0.01
	High	37.4		30.0 – 45.8	
12th July (+9d)	Low	27.9	2.02	10.0 – 38.8	<0.01
	High	35.8		27.0 – 45.0	
Average	Low	29.4	1.57	15.4 – 37.3	<0.01
	High	37.9		30.5 – 46.6	

The mean Predicted Transmitting Ability (PTA) for milk yield (kg) was calculated for both yield groups. This provides some indication of a cow's genetic merit as it shows the amount of yield potential that an animal is expected to pass on to its progeny. The PTA for milk yield was significantly higher for the high yielding group (data from Herd Genetic Report, August 2001) (data was not available for cows 236, 551 (low yielders) and 180 (high yielder) (Table 3.3b). This suggests that the significant difference in milk yield between the two groups is genetically determined. Both groups contained animals of a similar age (Table 3.3b). All cows were scored for body condition on a scale of one to five, to within 0.25 of a unit (Edmonson *et al.*, 1989), on three separate occasions, between 21st and 25th of June, by the same assessor (Table 3.1b). The low yielding cows had a higher mean body condition score and were further, on average, into lactation than the high yielding cows (P=0.02) (Table 3.3b).

Table 3.3b Details of mean values and ranges for body condition score (BCS), lactation number, days in milk (DIM) and Predicted Transmitting Ability (PTA) for milk yield (MY) for the experimental animals selected for high and low milk yields

Measure	Yield group	Mean	SED ±	Range	P-value
BCS	Low	3.09	0.070	2.67 – 3.58	0.003
	High	2.86		2.50 – 3.25	
Lactation number	Low	4	0.5	2 – 9	0.75
	High	4		2 – 8	
DIM	Low	238	14.3	164 – 305	0.02
	High	204		133 – 303	
PTA for MY (kg)	Low	126	58.5	-291 – 528	0.04
	High	249		6 – 484	

3.2.2b Conditions

For the duration of the experiment, both groups were kept together as part of the herd of 60 cows and were housed indoors between c. 14.15 and 08.30 h in a large straw-bedded shed (70 x 15 m) with *ad libitum* access to drinking water and a standard total mixed ration [grass silage (37.9%), maize silage (32.9%), concentrate pellets (Moulton balancer, Heygates Ltd, Northampton, UK) (14.0%), brewer's grains (13.8%), lucerne pellets (1.7%) and Megalac (0.3%)]. Feed was delivered once per day during morning milking. During periods of darkness, artificial lighting was provided at either end of the shed. For the rest of the day (c. 08.30 to 14.15 h), the cows grazed in a 4.74 ha paddock, with access to drinking water and Italian ryegrass pasture (*Lolium multiflorum*) (sown 1999, composed of equal mixes of: Atalja IRG, Ligrande IRG, Solid Hybred Tetraploid IRG, and Donergo Tetraploid IRG), which was kept at the recommended height of 8-10 cm (Chamberlain & Wilkinson, 1996). A concentrate allowance of 2 kg (Grassmaster 18, Heygates Ltd, Northampton, UK) was offered in the parlour to each cow per milking. Cows had been subjected to this

routine one week prior to this investigation. All cows were milked twice daily, between c. 05.15 and 06.30 h and c. 17.15 and 18.30 h. Individual cows were identified using coloured neck-collars, which were numbered on the top and sides from one to 10 in each of four colours (blue, green, red and yellow). Collars were assigned randomly to the selected cows as they left the milking parlour and entered a crush for fitting. Cows were allowed up to 2 h to become accustomed to the collars before the first set of observations began. All cows were in good health at the start of the study, *i.e.* they had no obvious signs of mastitis or clinical lameness.

3.2.3b 48 h Behavioural observations

Behaviour was recorded by three observers over a 48 h period commencing at c. 07.40 h on 21st June 2001. All observers were trained in the behavioural recording methods employed to reduce inter-observer error. The behavioural activities of all animals were recorded using instantaneous scan sampling (fixed interval time point sampling or point sampling) (Martin & Bateson, 1995) at 20 min intervals whilst outside and at 10 min intervals during the indoor period. These time intervals have been found to provide an accurate analysis of behavioural activities such as grazing (Hull *et al.*, 1960). The behaviours to be recorded were previously determined by spending one 24 h period with the cows, which also served to accustomise the cows to the observer's presence. Observations were conducted during all periods when the cows were not being managed as a group, for example, fed, cleaned out, milked or examined. These periods were from c. 09.00 to 16.00 h, *i.e.* from the end of morning feeding until preparation for afternoon milking, and c. 20.00 to 03.30 h, from the end of the evening feeding until the beginning of the morning milking. The 16 mutually exclusive ethological characteristics selected for analysis were: Feeding (grazing and

eating complete diet), drinking, ruminating (lying or standing), grooming self (lying or standing), grooming other cows (lying or standing), standing, walking, lying, sleeping (defined as neck being relaxed and eyes closed), mouthing housing furniture, rubbing housing furniture, nosing housing furniture and vocalising.

Cows were always observed in the same order. Binoculars were used to help identify individuals and minimise disturbance of the cows during the day, and a 1.5v torch was used at night. Observations ceased during movements, for example, from shed to field and shed to milking parlour. For each individual cow the total time spent in each activity was estimated by assuming that they had been performing the behaviour for the remainder of the 10 or 20 min period and multiplying the number of recordings of each behaviour by the number of scans in the 48 h period.

3.2.4b Rate of herbage grazing bites, and chewing rate and interbolus interval during rumination

The rate of biting herbage by each cow was recorded for a 90 sec period each day for four days commencing on 21st June 2001. The measurements took place approximately 1 h after the cows were moved into the paddock following morning milking. This had been determined as the best time from the behavioural study above as it provided the cows with sufficient time to settle and commence grazing. A bite was identified using the characteristic head movement and tearing sound that occurs when herbage is severed from the sward. The measurements were taken under similar weather conditions over the four days. Two observers were employed to ensure as short a time interval between observations on individual cows as possible. The measure of biting rate did not take into account periods of inactivity as counting was

restarted if grazing was interrupted for a period of 10 sec or longer (Phillips *et al.*, 1999). This methodology has been found to provide an accurate analysis of biting rate whilst grazing (Phillips & Leaver, 1985a). The number of chewing bites while ruminating was recorded during a 90 sec period for each cow on three occasions (Phillips *et al.*, 1999). This recording period included any time for bolus manipulation, *i.e.* regurgitation and swallowing. The interbolus interval was also recorded during this phase by recording the amount of time between the delivery of two consecutive boluses.

3.2.5b Rate and extent of sideways head movement during grazing

The rate of sideways head movement was recorded as a possible indicator of forage selectivity. This was determined by recording the number of sweeping movements of the head that crossed the central axis of the cow during a 2 min period (Rind & Phillips, 1999). In addition, the extent of sideways head movement was also measured by taking the furthest point reached before the head returned to the central position, for each head swing (Rind & Phillips, 1999). Observations were recorded from 3 m behind the observed cow using a hand held camcorder (Panasonic NV-RX9B). This distance was set to minimise observer disturbance. Four observations were taken for each cow over a period of five days between 27th June and 3rd July 2001 by one observer. Recordings took place on 27th, 28th and 29th June and on 2nd and 3rd July. Technical problems prevented observations taking place on the two days between these observation periods. Observations were taken between 09.30 and 12.30 h. Measurements commenced approximately 1 h after the cows were moved into the paddock following morning milking. This allowed sufficient time for the cows to settle and start grazing. The measurements were taken under similar weather

conditions over the five days. Measurements did not take into account periods of inactivity as counting was restarted if grazing was interrupted. On the second day of observations (28th June), it was necessary to move the cows into an adjacent field due to deterioration of sward conditions. Video recordings were later analysed and measurements of the rate and extent of sideways head movement were taken to the nearest 10 cm. A scale was produced for measuring the observations during playback by recording a 3 m length of tape and calculating the difference in magnification when presented on T.V. This process reduced the size of objects by 15 times, therefore the 3 m tape appeared as 20 cm when televised. One experimenter analysed all tapes.

3.3b Statistical analyses

All statistical analyses were performed using the 13th release of the Minitab statistical programme (Minitab, 2000). Milk yield data collected on the first day of study (*i.e.* 21st June) was used for all statistical analysis. For the behavioural observations, cows were split into two groups: high and low yielding. Each behavioural variable was tested for group differences by analysis of variance, providing that the values for each behaviour were normally distributed (as defined by the Anderson-Darling test, $P \geqslant 0.05$) and each comparative behaviour for both groups had equal variance (as defined by the F-Test if normally distributed, or Levene's if not normal, $P \geqslant 0.05$). Data not following a normal distribution were normalised by using logarithms of the original data, unless otherwise stated. If data were resistant to mathematical transformation to normalisation, or the variances were not homogenous, the original data were analysed by the Mann-Whitney test for non-parametric analysis. The Mann-

Whitney test was preferred over the Kruskall-Wallis and the Mood's Median as the former is an alternative to the two-sample t-test and is more robust, less easily influenced by outliers, and is a comparatively stronger test (Minitab, 2000). Where the data for those variables had greater than 50% zero values the 1-Sample Sign test was used separately for each group to examine whether the median value was statistically greater than zero (Snedcor & Cochrane, 1978). This provides a meaningful comparison between the two groups. Pearson's correlation coefficients were used between behaviour and production variables. A linear regression analysis was performed on statistically significant correlations.

3.3.1b 48 h Behavioural observations

The 48 h period was examined as one complete phase but was also split into indoor and outdoor periods.

As the behaviours observed were mutually exclusive, it was possible to combine behaviours to form new behavioural categories. For example, feeding and drinking behaviours were combined to form the new category 'ingestive behaviours'. A list of all the combined behaviours examined is shown under Table 3.5b in the results section.

For the entire 48 h period, all individual behaviours were normally distributed except for ruminating standing, sleeping, drinking and walking. It was not possible to improve the distribution of drinking behaviour by data transformation. For sleeping, it was necessary to use the square root of the original data to achieve a normal distribution. Mouthing, rubbing and nosing housing furniture, vocalising and all

grooming behaviours had too few observations for any useful statistical analyses to be carried out. The only combined behaviours not normally distributed, and also resistant to transformation, were stereotypic and grooming behaviours. Data for both ruminating and non-ruminating behaviours were not equally variated. Data for all other behaviours had homogenous variances.

All individual behaviours taking place indoors were normally distributed except for ruminating standing, sleeping, ruminating lying, drinking and walking. For sleeping, it was necessary to use the square root of the original data to achieve a normal distribution. Ruminating lying and drinking could not be normalised through transformation. Walking data had greater than 50% zero values. All individual behaviours were homogenous except for ruminating lying. The following combined behaviours, grooming, stereotypic, non-ruminating and ruminating were resistant to normalisation. Ruminating and non-ruminating behaviours were the only behaviours that were not equally variated.

The individual behaviours; lying, standing and walking when outside were resistant to normalisation. Only data for walking was not homogenous. All other behaviours followed a normal distribution. All combined behaviours except alertness, grooming and stereotypic were normal. Grooming and stereotypic behaviours contained greater than 50% zero values. Only alertness was resistant to normalisation, and not equally variated. No cows were observed sleeping outside.

An analysis of covariance (ANCOVA) (using the General Linear Model in Minitab) was performed for each behaviour (individual and combined) between treatment groups for milk yield, using days in milk and body condition score as covariates.

3.3.2b Rate of herbage grazing bites, and chewing rate and interbolus interval during rumination

It was not possible to improve the distribution of data for the number of grazing bites by mathematical transformation, thus the Mann-Whitney test was employed.

Both data for chewing rate and interbolus interval whilst ruminating followed a normal distribution.

3.3.3b Rate and extent of sideways head movement during grazing

Data for the rate and extent of sideways head movement were examined separately for movements to the left and right and also when combined (*i.e.* left + right). All data except for extent moved left and extent moved right were normally distributed. Measurements for the extent moved to the left were resistant to normalisation by mathematical manipulation. By taking the square root of the original data, it was possible to achieve a normal distribution for measurements of extent moved to the right. All data were homogenous.

3.4b Results

A probability value will be considered significant when $P \leqslant 0.05$. A probability value between 0.05 and 0.1 may be referred to as a trend or tendency.

3.4.1b Health status of animals during the study period

All cows maintained good health for the duration of the study except for one high and three low yielding dairy cows, which developed mastitis on 2nd July, which coincided with video observations for the rate and extent of sideways head movement during grazing. These cows had been observed three times (out of the required four) prior to the diagnosis of this condition, therefore these results were used for data analysis. One cow was omitted from this part of the study as she was particularly nervous and the observer was unable to get to the required 3 m distance to record the cow.

3.4.2b 48 h Behavioural observations

3.4.2.1b Individual behaviours

High yielding cows fed for longer over the entire 48 h period (P=0.01) (Table 3.4b). This was due to spending more time feeding outside (*i.e.* grazing) rather than inside (P=0.01, P=0.42, respectively). Time spent feeding over the two days was found to be positively correlated with milk yield (r^2=10.2, P=0.05) (Fig. 3.1b). There was also a non-significant trend for feeding time to increase with milk yield whilst indoors (r^2=9.1, P=0.06), but no significant correlation was found when outdoors.

High yielders spent, on average, 30 min less time lying outdoors in comparison to the low yielders (P=0.02) (Table 3.4b). High yielders also spent, on average, 23 min less time lying indoors, but this difference was not significant between the groups. There was no significant difference between groups for the time spent lying over the 48 h period. Lying was not correlated with milk yield over the 48 h period (P=0.16), indoors (P=0.16), or outdoors (P=0.66).

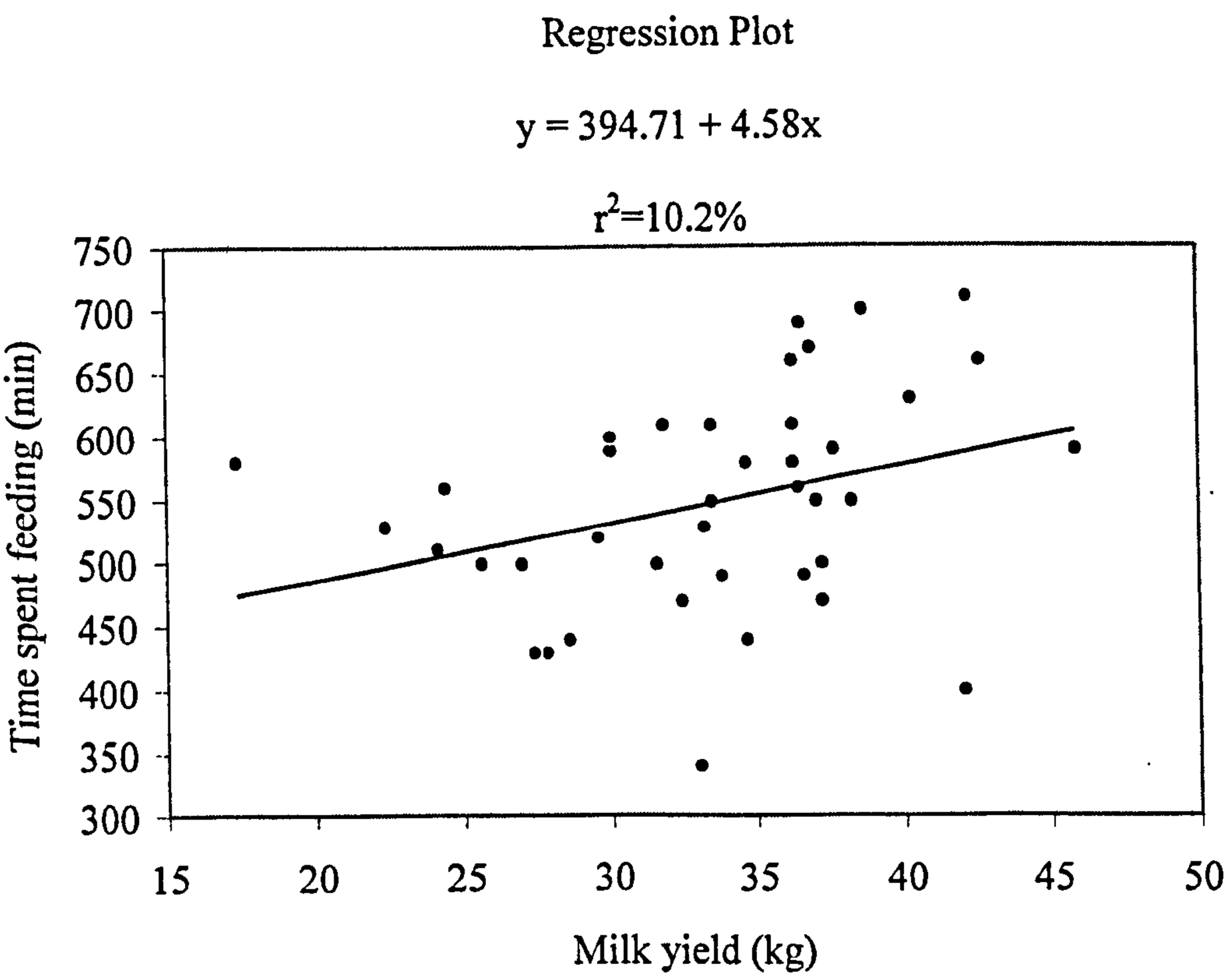


Fig. 3.1b Effect of milk yield on time spent feeding (min)

Cows were not observed to sleep outside. The high yielding cows spent, on average, 29 min less time sleeping indoors, but this difference was not significant. Time spent sleeping indoors was not significantly correlated with milk yield (Table 3.4b).

There was no difference between treatment groups for time spent standing during the 48 h period (P=0.41) (Table 3.4b). The high yielders spent approximately 10 min less time standing outside than low yielders (P=0.04), which was not correlated with milk yield. However, they spent more time standing indoors than the low yielders (P=0.02) and this was positively correlated with milk yield (r^2=9.6, P=0.05).

Table 3.4b The average time spent (min) in individual behaviours over the 48 h period, indoors and outdoors for each yield group. The significance level between treatment groups for each behaviour, the significance value for each behaviour when correlated with milk yield, the correlation coefficient and r^2 value for significant correlations

INDIVIDUAL behaviours *48 h Period*	Mean time (min) Lows	Highs	SED ±	P-value	Correlation coefficient	r^2 value	Correlation with milk yield P-value
Feeding	513	583	25.0	0.01	0.319	10.2	0.05
Lying	550*	495*	-	0.17	-0.224	-	0.16
Standing	161	178	20.3	0.41	0.208	-	0.20
Ruminating standing	82	123	20.8	0.07	0.293	-	0.07
Walking	45*	55*	-	0.25	-0.023	-	0.89
Drinking	20*	10*	-	0.32	-0.223	-	0.17
Ruminating Lying	645	603	30.7	0.18	-0.142	-	0.38
INDOORS							
Feeding (at trough)	208	221	15.9	0.42	0.302	9.1	0.06
Lying	500	477	29.1	0.43	-0.227	-	0.16
Standing	106	142	14.7	0.02	0.310	9.6	0.05
Sleeping	149	120	17.9	0.19	-0.259	-	0.11
OUTDOORS							
Feeding (grazing)	306	362	20.2	0.01	0.177	-	0.28
Lying	50*	20*	-	0.02	-0.072	-	0.66
Standing	50*	40*	-	0.04	-0.063	-	0.70

*Median value

During the 48 h period there was a tendency for the high yielding cows to spend more time standing ruminating than the low yielders (P=0.07). There was also a trend for ruminating standing to be positively correlated with milk yield (P=0.07) (Table 3.4b). There was no significant difference between the groups, or correlation with milk yield, for time spent standing ruminating both indoors and outdoors. When an analysis of covariance was performed, using days in milk as a covariate, this did not improve the significance of milk yield on standing ruminating over the 48 h period. However,

days in milk had a significant effect on the time spent standing ruminating during this time (P=0.05), and these variables were positively correlated (r^2=19.7, P < 0.01).

There was no significant difference between yield groups for walking, drinking and ruminating lying over the 48 h phase, inside or outside (Table 3.4b).

Lactation stage, when used as a covariate, had no significant influence with any other of the individual behaviours examined. Body condition score, when used as a covariate, had no effect on any of the above behaviours at the 0.05 or 0.1 significance level. There were no significant differences between treatment groups for any other individual behaviours examined.

3.4.2.2b Combined behaviours

Cows in the high yielding group spent significantly longer in ingestive behaviours, in comparison to the low yielding group, and there was a trend for this behaviour to be positively correlated with milk yield (P=0.08) over the 48 h period (Table 3.5b). The high yielding group also spent significantly longer in ingestive behaviours whilst outside, but not inside. This behaviour was not correlated with milk yield when the cows were either inside or outside (P=0.14; P=0.29, respectively).

High yielders spent less time in lying (P=0.01), leisure (P=0.02) and stereotypic (P=0.04) behaviours during the 48 h (Table 3.5b).

High yielding cows spent less time in lying behaviours when both inside and outside (P=0.04; P=0.01, respectively). Lying behaviours during the 48 h period, and when

inside, were negatively correlated with milk yield (r^2=13.7, P=0.02; r^2=17.8, P=0.01, respectively). Lying behaviours were, however, not significantly correlated with milk yield whilst outdoors. When an analysis of covariance was performed, using days in milk as a covariate, the effect of milk yield on lying behaviours over the 48 h period was non-significant, indicating that milk yield was not having a real effect on this behaviour. Days in milk did, however, have an effect on lying behaviours across this period (P=0.05) and was positively correlated (r^2=20.3, P < 0.01).

When outside, the high yielding cows spent less time in leisure behaviours (P=0.01), although there was no significant difference between groups when inside. However, at the significance level of 0.1, high yielders spent less time (51 min) in leisure activities when inside (P=0.09). Leisure behaviours were negatively correlated with milk yield during the 48 h period and when inside (r^2=13.1, P=0.02; r^2=13.7, P=0.02, respectively), but were not correlated when outside (P=0.33).

No difference between groups was observed for stereotypic behaviours when cows were inside (P=0.13). When the cows were outside, the high yielding cows received frequencies of zero for more than 50% of the individuals observed. Analysis of whether the median of zero was a true median of the population of each group, by the 1-Sample sign test, allowed comparison between the two groups. The probability of each group having a median greater than zero was significant for both groups (low yielders, P < 0.01; high yielders, P=0.03), therefore making no difference between the yield groups in the amount of time spent in stereotypic activities. Stereotypic behaviours were not significantly correlated with milk yield over the 48 h period or when inside or outside.

Table 3.5b The average time spent (min) in combined behaviours over the 48 h period, indoors and outdoors for each yield group. The significance level between treatment groups for each behaviour, the significance value for each behaviour when correlated with milk yield, the correlation coefficient and r^2 value for significant correlations

COMBINED behaviours *48 h Period*	Mean time (min) Lows	Highs	SED ±	P-value	Correlation coefficient	r^2 value	Correlation with milk yield P-value
Ingestive	532	597	25.1	0.01	0.284	-	0.08
Combined Lying	1362	1240	44.3	0.01	-0.370	13.7	0.02
Leisure	711	630	34.6	0.02	-0.362	13.1	0.02
Stereotypic	30*	10*	-	0.044	-0.103	-	0.53
Combined Standing	308	371	39.6	0.12	0.253	-	0.12
Alertness	206	234	23.9	0.28	0.167	-	0.30
Non- Ruminating	1480*	1495*	-	0.92	-0.074	-	0.65
Combined Grooming	25*	20*	-	0.35	-0.040	-	0.81
Combined Ruminating	730*	715*	-	0.92	0.075	-	0.65
INDOORS							
Ingestive	221	228	16.6	0.68	0.238	-	0.14
Combined Lying	1181	1113	31.1	0.04	-0.422	17.8	0.01
Leisure	648	597	29.7	0.09	-0.371	13.7	0.02
Alert	114	155	14.9	0.01	0.339	11.5	0.03
Combined Standing	164	228	25.3	0.02	0.374	14.0	0.02
OUTDOORS							
Ingestive	311	369	19.9	0.01	0.171	-	0.29
Combined Lying	182	128	1.0	0.01	-0.185	-	0.25
Leisure	60*	30*	-	0.01	-0.159	-	0.33
Combined Grooming	0 – 3[r]	0 – 2[r]	-	¶	-0.117	-	0.47

*Median value; r = range; ¶ Had greater than 50% zero values for combined observations. Used 1-sample sign test to analyse data (see text)

Key for combined behaviours:

- *Combined Ruminating*: Ruminating Lying + Ruminating Standing
- *Combined Standing*: *GOS + *GSS + Ruminating Stand + Standing + Walking
- *Combined Lying*: *GOL + *GSL + Lying + Ruminating Lying + Sleeping
- *Leisure*: Lying + Sleeping
- *Stereotypic*: Rubbing + *GSS + *GSL (Phillips, 1993; Fraser & Broom, 1997)
- *Ingestive*: Drinking + Feeding
- *Alertness*: Standing + Walking
- *Non-Ruminating*: All behaviours except those for Ruminating (above)
- *Combined Grooming*: *GOL + *GOS + *GSL + *GSS

*(G = Grooming; O = Others / S = Self; L = Lying / S = Standing)

There was no difference between yield groups in overall standing behaviours over the 48 h phase (P=0.12) (Table 3.5b). The high yielding cows spent longer in standing behaviours when indoors (228 versus 164 min, P=0.02), with no difference between the groups when outdoors (P=0.97). Standing behaviours were positively correlated with milk yield when inside (r^2=14.0, P=0.02), but not when outside (P=0.98), or over the 48 h (P=0.12).

There was only a difference observed between groups for alert behaviours when inside (P=0.01), which was also positively correlated with milk yield (r^2=11.5, P=0.03) (Table 3.5b).

There was only a significant difference for grooming activities when observed outside. This behaviour received observed frequencies of zero for more than 50% of the individuals observed. Comparison between the groups, by the 1-Sample sign test, examined whether the true median for the population of each group was one, or less than one. The frequency of observed grooming behaviours was not less than one for each animal in the low yielding group (P=0.07), while this was significant for the high yielding group (P=0.03). Grooming behaviours were not significantly correlated with milk yield.

There were no other significant differences between treatment groups for any of the other combined behaviours examined. Days in milk, when used as a covariate, had no significant effect on any other of the combined behaviours examined. Body condition score had no significant effect on any of the combined behaviours above when used as a covariate.

3.4.2.3b Behavioural correlations

Feeding was found to be negatively correlated with lying (r^2=17.4, P=0.01), combined lying behaviours (r^2=26.9, P<0.01), stereotypic type behaviours (r^2=10.6, P=0.02), grooming self whilst standing (r^2=9.5, P=0.05) and grooming (r^2=9.0, P=0.06). Stereotypic type behaviours were positively correlated with combined lying behaviours (r^2=14.3, P=0.02). Other behaviours, when correlated, were found to be significant but this was due to the recording method employed. For example, the amount of time spent standing was significantly negatively correlated with time spent lying. For this reason, such correlations were not included.

3.4.3b Rate of herbage grazing bites, and chewing rate and interbolus interval during rumination

The high yielding group had an increased bite rate during grazing but this was not significant at the 95% confidence level (P=0.07) (Table 3.6b). Bite rate was not significantly correlated with milk yield.

Table 3.6b Average rate of herbage bites during grazing and chewing rate and interbolus interval during rumination for the experimental animals selected for high and low milk yields

Behaviour	Mean		SED ±	P-value	Correlation coefficient	Correlation with milk yield P-value
	Lows	Highs				
Number of bites min^{-1}	53*	58*	-	0.07	-0.059	0.72
Number of chews min^{-1}	62	62	1.2	0.98	0.096	0.55
Interbolus interval (sec)	60	62	2.1	0.22	0.225	0.16

*Median value

There was no significant difference between the low and high yielding groups for the rate of chewing and the time between successive boluses whilst ruminating. Both

chewing rate and interbolus interval were not significantly correlated with milk yield (Table 3.6b).

3.4.4b Rate and extent of sideways head movement during grazing

There was no significant difference between the treatment groups for the rate or extent of sideways head movement to the left or right during grazing (Table 3.7b). In addition, significant difference between groups was observed when left and right measurements were combined for the extent of head movement. The high yielding group tended to reduce their rate of sideways head movements when the left and right measurements were combined (P=0.07). No movement, individual or combined, was significantly correlated with milk yield.

There was a significant negative correlation between the rate moved left and the rate moved right (r^2=18.4, P=0.01, Fig. 3.2b). There were no correlations between the extent moved left versus the extent moved right (P=0.15), and the rate moved left and right combined versus the extent moved left and right combined (P=0.13).

Table 3.7b Sideways head movement for the experimental animals selected for high and low milk yields

Head movement	Mean		SED ±	P-value	Correlation coefficient	Correlation with milk yield P-value
	Lows	Highs				
Extent Left (cm)	56*	57*	-	0.23	0.114	0.49
Extent Right (cm)	57	56	9.8	0.95	0.204	0.21
Extent Left + Right (cm)	110	114	4.4	0.40	0.202	0.22
Rate Left ¶	4	4	1.2	0.46	0.027	0.87
Rate Right ¶	4	4	1.2	0.24	-0.106	0.52
Rate Left + Right ¶	8	7	2.4	0.07	-0.071	0.67

*Median value; ¶Mean number of head movements per 2 min

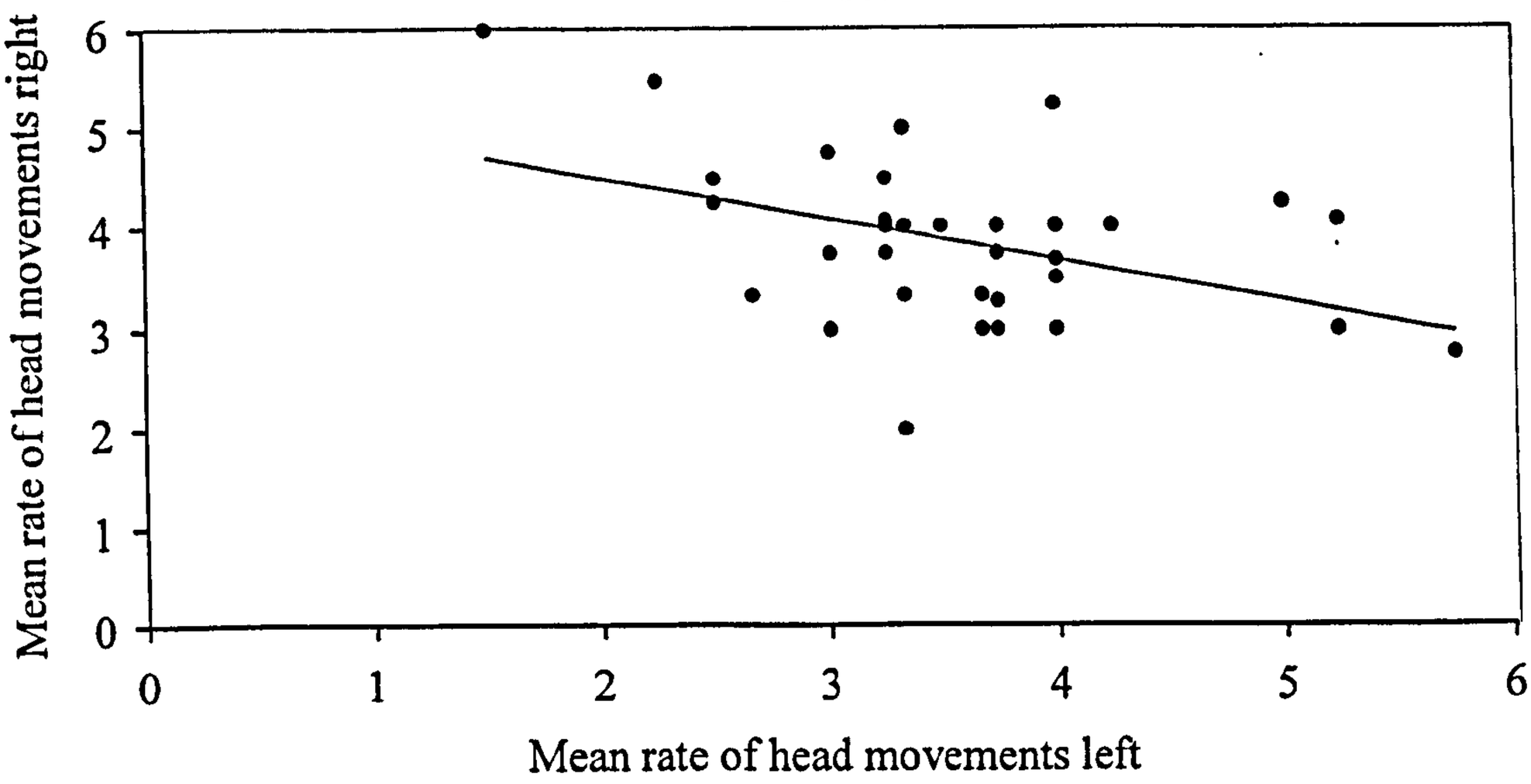


Fig. 3.2b Effect of the rate of sideways head movement to the left on the rate of sideways head movement to the right (Number 2 min^{-1})

3.5b Discussion

3.5.1b Individual behaviours

Of all the behaviours examined, the most notable impact of a high milk yield was on feeding. The amount of time spent feeding increased with milk yield, with the high yielding group spending significantly more time grazing but not eating conserved feed. This impact of milk yield on feeding time was expected as those animals with higher productive outputs have to satisfy their increased energy requirements in some way (Bao *et al.*, 1992). The high yielding group achieved this extra energy requirement by increasing their grazing time by approximately 1 h. Similar results have been found in several other studies (Hancock, 1953; Brumby, 1959; Stobbs, 1970; Lathrop *et al.*, 1988; Bao *et al.*, 1992; Gibb *et al.*, 1999). Extended grazing

times have also been observed in sheep which had increased nutritional demands (Penning *et al.*, 1995). Contrary to these results, O'Connell *et al.* (2000) found no difference in grazing time between high and low genetic merit dairy cows. However, it would seem that this was due to the small difference in milk yield between the two groups (3 kg d^{-1}), which was not great enough to have a significant impact on grazing time. It would appear that this extended grazing period is employed to increase grass intake, as these factors have been shown to be positively related (Hancock, 1953; Spedding, 1966; Gibb *et al.*, 1997).

Grazing time has been shown to increase following periods of fasting (Greenwood & Demment, 1988; Patterson *et al.*, 1998; Chillibroste *et al.*, 1997), and reduced intake rates (Manning, 1972), thus suggesting that the high yielding group were experiencing some degree of hunger, which in turn was motivating them to increase their forage intake. Hancock (1953), Baile and McLaughlin (1987), and Phillips (1993) have also suggested that the act of grazing itself is used to overcome the sensation of hunger, as it is hunger that motivates the cow to feed. Additionally, Manning (1972) has indicated that an extended grazing time is evidence of a higher feeding drive. Despite this, a correlation analysis revealed that time spent grazing was not related to milk yield. Other researchers have found similar results (Phillips & Leaver, 1986; Phillips and Hecheimi, 1989; Fuerst-Waltl *et al.*, 1999). This may be because cattle have a higher motivation to rest rather than feed when both activities are conflicting (Metz, 1985).

In order to increase the amount of time spent feeding the high yielder has to sacrifice the time spent performing one or more other activities. The behaviour most

significantly reduced whilst the cows were grazing was that of lying. There was also a negative relationship between these two behavioural activities over the 48 h period (P=0.01). Thus, it seems that for the high yielding cows to increase their forage intake they must forgo some of the time that would have been spent lying. This effect has been observed in other studies with cattle (Veris *et al.*, 1980; Fuerst-Waltl *et al.*, 1999; Fregonesi & Leaver, 2001), and also in other species. For example, Dunbar and Dunbar (1988) found that lactating female Gelada baboons (*Theropithecus gelada*) spent more time foraging and less time resting than those baboons with a smaller demand for food. Similarly, Muller-Scharze *et al.* (1982) established that white-tailed deer fawns increased their grazing time at the expense of performing other activities when they experimentally reduced their milk supply. It is difficult to determine whether this degree of reduced resting time had a significant impact on the cows' welfare. As cattle have revealed a high motivation to lie down (Metz, 1985) this may demonstrate not only the severity of hunger being experienced but also that the deprived resting period could be considered detrimental to their welfare. A reduced resting time can also have negative effects on other parameters such as milk production (Munksgaard & Lovendahl, 1993), hoof health and lameness (Singh *et al.*, 1993a; Leonard *et al.*, 1994; Faull *et al.*, 1996). To what extent this lying deprivation phase continues during the lactation period, and whether it is more intense at a particular stage of lactation is currently unknown. It was not possible to calculate the energy cost of this extended grazing time to determine whether this behavioural strategy was advantageous.

There was no observed difference between the groups for time spent feeding indoors. This may have been due to either the high yielder's motivation to rest exceeding that

to feed at this time, that they consumed the forage ration at a faster rate than the low yielding cows, or the extra time spent grazing was sufficient to achieve virtually complete energetic compensation. The experimenter attempted to measure the rate of intake for both yield groups but this was difficult to determine as the cows tended to 'lap' the ration rather than take obvious, countable bites.

The results also revealed a significant, though not substantial, reduction in the time spent standing for the high yielding group whilst outside. It would seem that this small difference is also linked to the extended grazing period, as no other behaviour was significantly reduced during this time. In contrast, the high yielding group spent more time standing indoors and there was a non-significant trend for this to increase with milk yield. A likely explanation is that the cows were actually feeding but were observed as standing. This may be supported by the non-significant trend (P=0.06) for feeding time to increase with milk yield indoors. Phillips and Leaver (1986) also found that cows spent longer standing inside when fed a silage supplement overnight.

A correlation analysis revealed a weak but positive trend between the time spent ruminating standing and milk yield over the 48 h period only (P=0.07). It is possible that the observer confused this with feeding behaviour. The animal may have been masticating food, with its head raised, between bites. The high yielding group also had a tendency to spend more time ruminating standing over the 48 h period, which has also been observed by Phillips and Hecheimi (1989), and O'Connell *et al.* (2000). This extra ruminating time may have increased the digestibility of the forage thus enabling them to achieve a higher forage intake (O'Connell *et al.*, 2000), or may have been required to digest the additional forage consumed.

3.5.2b Combined behaviours

The high yielding cows spent longer in ingestive behaviours during the 48 h. This was due to the extended foraging period rather than time spent drinking. Combining both feeding and drinking behaviours reduced the significance value between the yield groups compared with feeding alone. This is because the high yielding cows spent less time drinking in comparison to the low yielding group, but not significantly.

The high yielding group spent significantly less time in lying behaviours across the entire 48 h period, inside (-68 min) and outside (-54 min). Thus, the high yielding cows spent over 2 h less time resting during the study period. This was primarily due to a reduction in the performance of leisure activities (*i.e.* sleeping & lying), which accounted for approximately three quarters of this time. This further highlights the impact of an increased grazing period on the time available to rest outside. Both time spent in lying and leisure activities decreased with increasing milk yield for the high yielding group whilst inside. The reason for this seems to be due to the non-significant trend for both feeding and standing time to increase during this period, as discussed earlier.

Combined standing behaviours were significantly greater for the high yielding group and positively correlated with milk yield when inside. It would appear that ruminating standing and standing were having the largest effect on the combined standing behaviours as only minimal differences were observed between the groups for individual grooming activities and walking behaviour. The increased amount of time

spent in alert behaviours indoors for the high yielding group is also likely to be connected to the longer time spent standing.

The high yielding group spent less time performing stereotypic type behaviours over the 48 h period. As there was a significant negative correlation between feeding and stereotypic type behaviours, this suggests that the high yielding cows may not have engaged in such activities due to their extended foraging period. This alludes to a link between the time available to rest and the carrying out of stereotypic type behaviours, which is confirmed by the positive relationship between these behaviours. This is additionally supported by the high yielding cows spending less time in grooming activities when outside (*i.e.* where they spent longer grazing), the negative correlation between time spent feeding and grooming, and the negative correlation between the time spent feeding and grooming self whilst standing.

3.5.3b Rate of herbage grazing bites and chewing rate and interbolus interval during rumination

The high yielding group had a tendency to increase biting rate during grazing. Comparable results have been found in several other studies with dairy cows (Gibb *et al.*, 1999; O'Connell *et al.*, 2000) and in sheep that had increased nutritional demands (Penning *et al.*, 1995). An increased biting rate has been associated with a greater appetite as studies that have manipulated the intake levels of dairy cows or subjected them to a period of fasting have observed similar findings (Manning, 1972; Greenwood & Demment, 1988; Dogherty *et al.*, 1987 & 1989; Patterson *et al.*, 1998). This provides complementary evidence to illustrate the greater need for high yielding cows to increase intake rates during grazing. Phillips and Leaver (1985b) have

suggested that there is an upper limit to grazing biting rate of 60-70 bites min^{-1}. As the high yielding group were approaching this limit (mean = 57.1 bites min^{-1}) there was little scope for them to increase this behaviour and thus be significantly different from the low yielding cows at the 0.05 probability level. Fuerst-Waltl *et al.* (1999) witnessed no effect on biting rate for high yielding cattle. This may have also been due to the cows reaching this upper biting limit.

The high yielding group neither chewed faster nor increased the frequency of boluses during rumination, thus the rate of ruminating activity was similar to the low yielding group. It may have been unnecessary to increase the rate of these activities as the extra time spent ruminating adequately catered for the increased forage intake. Alternatively, the cow may have reduced the physical processing of the forage (Gibb *et al.,* 1999) to cope with this additional demand.

3.5.4b Rate and extent of sideways head movement during grazing

No difference was observed between the yield groups for the rate and extent of sideways head movement during grazing. Dogherty *et al.* (1987) proposed that fasted cattle may increase intake rates by being less selective. The results from this study do not confirm this suspicion from a behavioural perspective for the high yielding group. Geenwood and Demment (1988), on examining diet quality in fasted cattle, found that they could increase their intake rates without being less selective, thus supporting the findings presented here. Likewise, Sidahmed *et al.* (1977), and Chacon and Stobbs (1977) using cattle and Langlands (1967), and Hodgeson (1981) using sheep found no effect of fasting on diet quality. In contrast, as Patterson *et al.* (1998) increased the duration of fasting in cattle, the proportion of time spent in forage selection

progressively decreased. However, this was only observed after at least 6 h of fasting and this level of induced hunger may not be equivalent to that being experienced by the high yielding group in this experiment. In addition, the high yielders increased grazing time may have allowed them to maintain forage selectivity.

Interestingly, there was a negative relationship between the rate of head movements to the left with rate moved to the right during grazing for both the high and low yielding cows combined. This provides some indicator of handedness, *i.e.* a preferred grazing side. The underlying reasons behind this are unknown, but it was not shown to be related to milk yield.

In conclusion, it would appear that there were two strategies that the high yielding dairy cows adopted to increase forage intake in an attempt to satisfy their increased appetites. These were, to increase the amount of time spent grazing, and to increase biting rate. An additional method is to increase dry matter intake per bite (Chacon & Stobbs, 1977; Patterson *et al.*, 1998), but this was not measured. As similar results to those found in this study have been demonstrated with fasted cattle, it is reasonable to propose that the high yielding dairy cows used were experiencing some degree of hunger. The degree to which this was impacting on their welfare remains undetermined. It appears that in order to increase grazing activity the amount of time available for rest has to be reduced. The degree to which this impacted on the cows welfare is also unknown, but is presumed to be significant as cattle have a high motivation to rest (Metz, 1985). The small difference in milk yield between the two yield groups (mean of 8.5 kg d^{-1}, Table 3.2b) may have been responsible for the non-significant differences observed. Also, the recording method employed may not have

been sensitive enough to accurately predict the time spent in those behaviours that are typically of a short duration (*e.g.* grooming).

From a review of the literature, it would seem that there are two possible ways to reduce the extended grazing time observed for the high yielding group and thus increase the amount of time available for resting. Both methods aim to increase the rate of dry matter intake. The first is to offer supplementary rations, which have been shown to reduce grazing activity (Sarker & Holmes, 1974; Leaver, 1985; Phillips, 1993; O'Connell *et al.*, 2000) and increase resting time (Phillips & Leaver, 1986; Munksgaard *et al.*, 2001). Supplementation can be achieved by offering, for example, additional concentrates (O'Connell *et al.*, 2000; Munksgaard *et al.*, 2001), hay or forage (Phillips & Leaver, 1986). O'Connell *et al.* (2000) suggested that a concentrate supplement is necessary to meet the energy requirements of grazing dairy cows yielding in excess of 25 kg d^{-1}. As forage supplementation was provided in this experiment, grazing times would be expected to be higher in both treatment groups if this was not available. This would be to the further detriment of resting time for the high yielding group. The second method is to increase sward surface height. This enables the cow to achieve a greater bite depth therefore increase bite mass and thus dry matter intake rate (Laca *et al.*, 1992; McGilloway *et al.*, 1997; Gibb *et al.*, 1999; O'Connell *et al.*, 2000). Gibb *et al.* (1999) observed a significant decrease in grazing times with increases in sward heights by as little as 4 cm (*i.e.* from 5 to 9 cm). Intake rates remained the same as those when cows were grazed on a shorter sward but were achieved in a shorter period.

As it is the difference in milk yield between the two groups selected that dictates the scale of the variation in their grazing times, it is not possible to predict at what point rest deprivation starts to impact on a cow's welfare by comparison. It is therefore important to establish a milk yield baseline, which could be used to compare and make a welfare judgement about the resting times of a higher yielding group. As factors such as the provision of supplements can affect these measurements they should be given consideration. Further research should aim to determine either how much resting time a cow requires to maintain full health and welfare, or the level of rest deprivation that starts to affect health and welfare. This information can then be used to predict optimum milk yield levels, which enable the cow to cope under both extensive and semi-intensive environments before her welfare is compromised. A semi-intensive management system may provide the best compromise between the farmers requirement to maximise milk yields and the cows requirement for an open environment.

4. The motivation of high and low yielding dairy cows for supplementary concentrate feed

4.1 Introduction

It is becoming increasingly difficult to meet the energy requirements of high yielding dairy cows as milk yields rise (Kamphues, 1998). This is partly due to the way in which artificial selection for greater milk yields has taken place. Emphasis on the selection for improved yields has neglected the selection of those physical characteristics designed to cope with this greater physiological demand. For example, Kamphues (1998) stated that in high yielding dairy cows, feed intake capacity has not increased to the same extent as milk production. This has produced cows with high milk yields that are unable to consume sufficient amounts of roughage to maintain full health whilst sustaining a high level of production. Consequently, these cows are in a prolonged state of negative energy in early lactation, which makes them more susceptible to health problems such as emaciation, dystocia, acetonaemia and fatty liver disease (Gearhart *et al.*, 1990). In an attempt to meet this demand an increased proportion of high energy concentrates have to be included in the diet. O'Connell *et al.* (2000) stated that it is necessary to provide a concentrate supplement to cows yielding greater than 25 kg milk d^{-1}, as forage alone is insufficient to meet their nutrient requirements. However, this additional concentrate allowance can present further problems by unbalancing the roughage-to-concentrate ratio and thus predispose the dairy cow to various other health troubles (Kamphues, 1998), for example, acidosis and laminitis. Flachowsky and Lebzien (1997) have further stated that even when high amounts of concentrate are fed, it is difficult to cater for the energy requirements of cows producing in excess of 40 kg milk d^{-1}.

This evidence may suggest that the provision of a standard forage ration does not satisfy the appetite of high yielding dairy cows. This may result in high yielding dairy cows suffering from a constant hunger in relation to lower yielding cows. If this were true, the higher yielding cows may demonstrate the need for an additional concentrate allowance in comparison to lower yielding cows, if given the opportunity. This suggestion can be explored with the use of operant conditioning techniques, which can provide a useful methodology to measure the demand for various resources (Dawkins, 1983a). Therefore, this procedure was adopted to measure the appetite of high and low yielding cows maintained on a standard commercial total mixed ration. Such a test is useful as the amount of work done is representative of the level of deprivation an animal receives (Robinson, 1998). The assumption underlying such tests is that the more an animal wants or needs something the harder it is prepared to work in order to obtain it (Dethier & Stellar, 1970). The test requires the animal to work by pressing a lever with its nose to gain access to a concentrate reward. The cows were exposed to a progressive-ratio three schedule of food delivery, which required the animal to press the lever in increasing multiples of three in order to obtain each successive food reward. Breaking points, *i.e.* the largest ratio completed before responding ceases, were recorded for each cow. These were used to compare work rates between the high and low yielding groups. In addition, post-behavioural testing was conducted on each animal to reveal whether there were any differences between groups in their motivation to subsequently consume forage.

4.2 Materials and Methods

4.2.1 Animals

The experiment, which was conducted between 27th August and 28th September 2001, used cows from the Holstein-Friesian dairy herd of Moulton College, Northampton. Thirteen pregnant, lactating and experimentally naive dairy cows were selected and split into two yield groups: Six low yielding and seven high yielding, with mean milk yields of 14.2 kg d^{-1} (s.e. 2.48) and 26.4 kg d^{-1} (s.e. 1.13) respectively ($P<0.01$), based on pre-experimental National Milk Records (NMR) taken on 14th August for group one, and 12th September for group two (Table 4.1). For ease of testing, and replication, the cows were divided into two test groups: The first group consisted of four cows from each yield group, the second contained two low yielders and three high yielders (Table 4.1).

Table 4.1 Characteristics (group number, cow number, milk yield (MY), days in milk (DIM), lactation number and body condition score (BCS) of the experimental animals in the high and low yielding groups

Low yielding group						High yielding group					
Test group	Cow No.	MY (kg)	DIM	Lact. No.	BCS	Test group	Cow No.	MY (kg)	DIM	Lact. No.	BCS
1	74	5.4	395	3	2.50	1	549	23.4	310	3	2.75
1	47	8.2	335	3	3.50	2	112	24.6	255	2	3.25
1	564	15.0	340	3	3.25	2	19	24.8	295	2	3.00
2	561	17.0	304	4	3.50	2	52	25.2	334	3	3.25
1	93	18.8	352	2	3.00	1	95	26.4	371	3	3.00
2	51	20.6	330	2	2.75	1	138	28.4	338	3	3.00
						1	21	32.2	376	3	2.75
Mean		**14.2**	**342.7**	**2.8**	**3.08**			**26.4**	**325.6**	**2.7**	**3.00**

All cows were scored for body condition on a scale of one to five, to within 0.25 of a unit (Edmonson *et al.,* 1989), at the beginning of the experiment by the same assessor. The low yielding cows had a mean body condition score of 3.08 (s.e. 0.17), compared with 3.00 (s.e. 0.08) for the high yielding cows (P=0.642). Both groups were balanced for stage of lactation, age and stage of pregnancy (Table 4.2).

Table 4.2 Details of mean or median values and ranges for body condition score (BCS), lactation number, days in milk (DIM) and days to parturition (DTP) for the experimental animals selected for high and low milk yields

Measure	Yield group	Mean	SED ±	Range	P-value
BCS	Low	3.08	0.19	2.50 – 3.50	0.642
	High	3.00		2.75 – 3.25	
Lactation number	Low	3*	-	2 – 4	0.89
	High	3*		2 – 3	
DIM	Low	343	20.3	304 – 395	0.43
	High	326		255 - 376	
DTP	Low	77	15.1	50 – 105	0.12
	High	103		57 – 142	

* Median value

4.2.2 Experimental conditions

Prior to the study the animals were housed as a group, together with 46 other cows, indoors with straw bedding. For the duration of the experiment, the selected cows were housed together in a large straw-bedded pen (18 x 9 x 4 m) (Length x Width x Height) with constant access to drinking water and a standard commercial total mixed ration [grass silage (37.9%), maize silage (32.9%), concentrate pellets (Moulton balancer, Heygates Ltd., Northampton, UK) (14.0%), brewer's grains (13.2%), lucerne pellets (1.7%) and Megalac (Volac Ltd., Royston, Herts UK) (0.3%)] [Crude protein (18.1%), Metabolizable energy (12.2 MJ kg DM)]. Feed was delivered once

per day whilst the animals were being milked in the morning. All cows were milked twice daily between c. 06.30 and c. 07.15 h and c. 15.30 and 16.15 h. During the morning milking a restricted concentrate (Grassmaster 18, Heygates Ltd., Northampton, UK) allowance of 250g was provided per cow. In the afternoon and days when the cows were not tested, the allowance was 2 kg. Individual cows were identified using their freeze brand number. All cows were in good health at the start of the study, *i.e.* they had no obvious signs of mastitis or clinical lameness.

4.2.3 Apparatus

The experimental apparatus (operant device) was placed in a separate, large, straw-bedded pen (test pen) (4 x 9 x 4 m) (L x W x H) adjacent to the pen where the cows were being housed. The device was a modified, commercially available, nose-operated, cast iron, single cattle drinker bowl (6 x 20 x 13 cm) (L x W x H) (Fisher Drinkers, Birmingham). A length of plastic half-gutter-piping (16 x 18 x 5 cm) (L x W x H) was bolted to the bottom of the bowl. A spring activated, cast iron, nose operated lever was situated in the centre of the bowl (10 x 5.5 cm) (L x W). The lever was covered with yellow tape as this was thought to enhance its visibility to the dairy cow. When depressed, the lever made an electrical connection to sound a bell (Friedland Battery Powered Doorbell Kit D937), which acted as an auditory signal to the observer, and the cow, indicating the number of times the lever had been depressed. A minimum response force of 8 N was required for lever operation and to sound the bell. This was determined by putting the device on its back and placing an empty plastic bottle on the lever. The bottle was gradually filled with water until it was sufficiently heavy enough to depress the lever and sound the bell. The bottle was then weighed in kg and converted into a force in N. The device was mounted centrally

on a metal hurdle, 90 cm above the ground. This enabled good visibility and easy operation of the device by the cow. A bucket was situated on the ground to the right of the operant device into which a food reward was manually delivered via a chute (plastic gutter pipe) by the observer. No other food was available to the cow within the test pen.

4.2.4 Training

Both test groups required eight days of training, which took place between 14th and 24th August for group one, and between 10th and 19th September for group two. Both test groups started with 10 cows; five high yielding and five low yielding. Those cows (one low yielder and one high yielder in group one, and three low yielders and two high yielders in group two) that had not learnt the test after eight days, and were perceived as being unable to learn the test with further training, were eliminated from the group. This resulted in the 13 test cows: Six low yielding and seven high yielding. The trial cows were made familiar with the experimental conditions by allowing them to wander in and out of the test pen for 1 h prior to the start of the training period.

The cows were accustomed to the handler by spending approximately 4 h per day for two days offering each cow 360g of dairy pellets and engaging in positive physical contact. When the cows no longer reacted negatively to being touched, they were considered ready and accustomed.

The experimental cows were trained individually in daily 30 min sessions to operate the device (by pressing the lever) on Fixed Ratio (FR) schedules of reinforcement for food rewards. Initially the cows were encouraged to investigate the device by placing

a small number of standard dairy nuts (Heygates Ltd., Northampton, UK) [Ingredients: Wheatfeed (20.5%), Rape ext. (15.5%), Vegetable oil (15.0%), Sunflower ext. (13.0%), Wheat (10.7%), Palm Kernel exp. (9.0%), Maize gluten (8.0%), Molasses (8.0%), Wheat flour (5.0%), Citrus Pulp (4.0%), Limestone (2.1%), Minerals (1.5%), Wafolin (0.8%), Salt (0.4%)] [Protein (16.0%), Fibre (10.0%), Ash (8.5%), Oil (4.8%)] [Energy (12.4 MJ kg DM)] (pellets were cylindrical: aprox. 1.5 x 0.4 cm) onto the gutter piping attached to the device. Each time the cow attempted to eat the pellets, therefore muzzling the lever, the buzzer was sounded and 165g of dairy nuts were delivered into the feed bucket. The cow then received one food reward each time the lever was depressed. After three rewards, the cow had to press the lever twice to receive the same reward. After a further two rewards the cow had to press three times per reward. This regime continued with the number of presses required per reward increasing in multiples of two after every second reward until the experimental cow received one reward for nine presses (Table 4.3). When each cow was capable of achieving this they were considered fully trained for the experiment.

Table 4. 3 Training regime

No. of presses required per reward	Number of times repeated
1	3
2	2
3	2
5	2
7	2
9	1

Table 4.4 shows the number of 30 min training sessions required by each cow to learn the experiment. Learning, in this case, was taken to mean the amount of time required to make the association between lever pressing and food delivery.

Table 4.4 Number of training sessions required by each cow to learn the experiment

Low yielders			High yielders		
Test group	**Cow number**	**Number of sessions**	**Test group**	**Cow number**	**Number of sessions**
1	74	6	1	549	6
1	47	6	2	19	6
1	564	6	1	95	5
1	93	5	1	138	5
2	561	5	2	112	5
2	51	5	2	52	5
			1	21	4

Table 4.4 demonstrates that the majority of the training period was required to make the association between lever pressing activity and reward delivery. Once this was learned, it only took a further two or three sessions to complete the training schedule. There was no significant difference between the two yield groups and between the test groups in the amount of time taken to learn the experiment ($P=0.43$, $P=0.61$, respectively). The success of this short training period was probably due, in part, to training the animal in a familiar environment and allowing the test animal to have visual contact with the other group members if required. There was also the opportunity for the test cow to engage in physical contact with other cows, although no cow was observed to take advantage of this.

The restricted session time, *i.e.* the amount of time available to work for rewards, was employed as an attempt to prevent the occurrence of habituation (the waning of a response). It was also expected that the cows would learn that they only had a limited

amount of time to work for the concentrate rewards and would therefore aim to make best use of this time. This time allowance also allowed all cows to be tested each day.

4.2.5 Experiment

The trial period for group one ran for 12 consecutive days commencing on 27th August. Group two commenced on 20th September and ran for a slightly shorter period of nine consecutive days due to unforeseen alterations in farm management practices. Tests took place after morning milking and commenced between 07.30 and 07.45 h.

Individual cows were given the opportunity, in daily test sessions of 30 min, to work on a Progressive Ratio (PR) schedule of reinforcement for access to 0.33 kg of dairy nuts (the same as those used for training). This methodology required the cow to perform an increasing number of responses in order to obtain each successive reward. The reward value was selected to prevent acidosis by assuming that the cows would work for approximately 12 rewards (PR33), therefore allowing a total reward value of 4 kg of dairy nuts per daily test session. This assumption was based on work rates during the training period. The number of lever presses required to obtain each subsequent reward was increased by a fixed increment of three, with the first reward being delivered after one press, the second after three, the third after six, the fourth after nine, and so on. This regime was designed to gradually increase the amount of work required to receive each successive reward. This methodology has been shown to vary reliably according to changes in food deprivation (Hodos, 1961; Robinson, 1998) and is a more sensitive measure than Fixed Ratio (Lawrence & Illius, 1989). The PR increment selected has been shown to be of little importance to the results

obtained as it is the degree of satiation that determines the breaking point rather than the increment size (Robinson *et al.*, 1995).

Cows were tested in alternating milk yield order, which was determined during the training period by allowing the cows to enter the test pen freely. Cows were tested at the same time and in the same order each day to ensure that they were in a similar physiological state at the start of each period.

It was necessary for the test cow to press the lever the required number of times continuously. If the cow ceased to press the lever for a period of greater than 30 sec, the number of accumulated presses up to that point were forfeited and counting started again from zero. The point at which the cow ceased responding to the increasing ratio was used as a measure of strength of feeding motivation. Extinction was defined as occurring if the cow did not respond for a 5 min period. This time limit was determined during the training period, as the cows would rarely start responding again after this period of time had elapsed. The cow would then be returned to the housing area and the next cow would begin the trial.

4.2.6 Observations

The observer was situated directly behind the operant device and monitored the experimental cow via two small slits cut into the wooden partition. The wooden screen separated the observer from the test animal and therefore minimised the transmission of any possible visual and odour cues. No artificial illumination was required due to the open plan of the building allowing sufficient natural lighting.

At the end of each test session a 'breaking point,' *i.e.* the largest ratio completed before responding ceased for five consecutive minutes, was determined for each cow. The observer also made records of the time of delivery for each reward, and the total amount of time each cow spent in the test pen (including the 5 min non-responding time if relevant). The delivery time for each reward included the consumption time of the previous reward and the time taken to press the lever the required number of times to obtain the next reward. The observer also recorded the behaviour of each cow over a 20 min period on return to the housing pen post-testing. The behavioural activities were recorded using instantaneous scan sampling (fixed interval time point sampling or point sampling) (Martin & Bateson, 1995) at 2 min intervals. Hull *et al.* (1960) have found this methodology provides an accurate analysis of behavioural activities. The behaviours to record were selected by observing the cows during the post-testing period on previous test days. Six mutually exclusive ethological characteristics were selected for analysis: Drinking, feeding, lying, standing, ruminating lying, and ruminating standing. Group one was observed for three consecutive days commencing on the tenth day of the experiment. Group two was monitored for five consecutive days commencing on the fourth day of testing. For each individual cow the total time spent in each activity was estimated by assuming that they had been performing the behaviour for the remainder of the 2 min period and multiplying the number of recordings of each behaviour by the number of scans in the 20 min period.

4.3 Statistical analyses

All statistical analyses were performed using the 13th release of the Minitab statistical programme (Minitab, 2000). Milk yield data collected on 12th September was used for all statistical analysis.

4.3.1 Breaking points, amount of time in test pen, mean time taken to receive a reward, and the time taken to receive the first and fourth rewards

For each measurement the General Linear Model (GLM) was used to determine whether there was any significant difference between replications for each yield group. No significant differences were detected for any of the measures, and therefore data for both replications were included in all statistical analyses. The GLM procedure was selected over the two-way analysis of variance, as the experiment was unbalanced due to a different number of animals in each replication.

Each measure was tested for yield group differences by analysis of variance (ANOVA), provided that the values for each yield group were normally distributed (as defined by the Anderson-Darling test, $P \geq 0.05$) and those variables to be compared had equal variances (as defined by the F-Test if normally distributed, or Levene's if not normal, $P \geq 0.05$). All data, for all of the measures, were normally distributed and had equal variances.

Pearson's correlation coefficients were determined between each measure and milk yield (MY), days in milk (DIM), body condition score (BCS) and days to parturition (DTP). Breaking points were also correlated with the amount of time spent in the test pen. A linear regression analysis was performed on statistically significant correlations.

An analysis of covariance (ANCOVA) (using the GLM) was performed for each measure between yield groups for MY, using DIM, BCS, and DTP as covariates.

The mean time taken to receive the fourth reward was used as it represents the minimum number of rewards that all cows received, except for one high yielding cow (no. 95). Cow 95 only received a maximum of three rewards for the whole of the test period and was omitted from the analysis for this measure.

Intake rates (g min^{-1}) for the concentrate reward were also tested for yield group differences by ANOVA, providing that the values for each yield group were normally distributed (as defined by the Anderson-Darling test, $P \geqslant 0.05$) and those variables to be compared had equal variances (as defined by the F-Test if normally distributed, or Levene's if not, $P \geqslant 0.05$). Intake rates were also correlated with milk yield.

4.3.2 Mean breaking points achieved per day – by yield group

The Anderson-Darling test was used to ensure that the values for each yield group were normally distributed. Both data sets were distributed normally. For each yield group the Pearson's correlation coefficient was used between the mean breaking points achieved each day and the first nine test days. The mean breaking points achieved were tested for yield group differences by ANOVA, using the results obtained for the first nine test days, days one to five and days six to nine. Only the first nine days were used, to provide the same time period for comparison between all cows. A visual inspection of a linear regression analysis for the high yielding group indicated that the data points were not evenly spread around the regression line, implying that the relationship between the variables may not have been linear. An exponential regression analysis was therefore performed on the data, but this gave a less good line of best fit. Thus, the mean breaking points achieved for the high

yielding group were best expressed using the linear regression. A correlation and linear regression were also performed for both the high and low yielding groups using the breaking points achieved for the first five days (days one to five) and the last four days (days six to nine) to examine any further trends during the experiment.

4.3.3 Mean breaking points achieved per day – for individual cows

Each cow's data was checked for normality using the Anderson-Darling test. All data, for each cow, were normally distributed except for cows 74 and 95. The data, for both cows, were resistant to mathematical transformation to normalisation. Spearman's rank-order correlations (a non-parametric alternative to the Pearson's correlation coefficient) were conducted for these cows using the breaking points achieved each day versus the number of experimental days each cow was tested for (12 days for cows in group one, and nine days for cows in group two). For each of the other cows, Pearson's correlation coefficients were conducted between the same variables. A linear regression analysis was carried out on statistically significant correlations.

4.3.4 Mean time to receive each successive reward

Data values for each cow were tested for normality using the Anderson-Darling test. All data were normally distributed. For each cow and yield group, the mean amount of time taken to receive each successive reward (*i.e.* time taken to receive the first, second, third, *etc.* reward) was correlated against the number of rewards received, using Pearson's correlation coefficients. Linear regression analyses were performed on statistically significant correlations.

4.3.5 Behavioural observations

For feeding, standing and drinking behaviours the General Linear Model (GLM) was used to determine whether there was any significant difference between replications for each yield group. No significant differences were detected for any of these behaviours. Data for both replications were therefore taken together in all statistical analyses for each behaviour. No cow in group two was observed lying, consequently only the high and low yielding cows in group one were used for data analysis. No cow was observed ruminating.

Each behaviour was tested for yield group differences using ANOVA, provided that the values for each yield group were normally distributed (as defined by the Anderson-Darling test, $P \geqslant 0.05$) and those variables to be compared had equal variances (as defined by the F-Test if normally distributed, or Levene's if not normal, $P \geqslant 0.05$). All the behaviours examined were normally distributed and had equal variances except for drinking behaviour, which was not normally distributed. Drinking behaviour was normalised using logarithms of the original data. Pearson's correlation coefficients were used between each behaviour and MY, DIM, BCS and DTP. A linear regression analysis was performed on statistically significant correlations. An ANCOVA (using the GLM) was performed for each behaviour between yield groups for MY, using DIM, BCS, and DTP as covariates.

4.4 Results

A probability value will be considered significant when $P \leqslant 0.05$. A probability value between 0.05 and 0.1 may be referred to as a trend or tendency.

4.4.1 Health status of animals during the study period

All cows maintained good health for the duration of the study, *i.e.* they had no obvious signs of mastitis or clinical lameness.

4.4.2 Breaking points

Table 4.10 shows the mean breaking points for individual cows. The GLM did not indicate any differences in the breaking points achieved between replications for each yield group (P=0.73). There was no significant difference between yield groups in the mean breaking points achieved (Table 4.5). Breaking points were not significantly correlated with milk yield. The number of days in milk (DIM), body condition score (BCS), and number of days to parturition (DTP) had no effect on breaking points when used as covariates in an analysis of covariance (P=0.15; P=0.69; P=0.36, respectively). The use of these covariates did not significantly improve the effect of milk yield on the breaking points achieved (P=0.14). There was no correlation between breaking points and BCS or DTP (P=0.36; P=0.37, respectively). However, there was a tendency for a negative correlation between breaking points and DIM (r^2=23.0, P=0.10) (Fig. 4.1).

Table 4.5 The mean breaking points achieved and the mean time spent in the test pen for the experimental animals selected for high and low milk yields

Observation	Mean		SED ±	P-value	Correlation coefficient	Correlation with milk yield P-value
	Lows	Highs				
Breaking point	18	14	4.6	0.44	-0.067	0.83
Time in pen (min)	21	19	2.6	0.40	-0.065	0.83

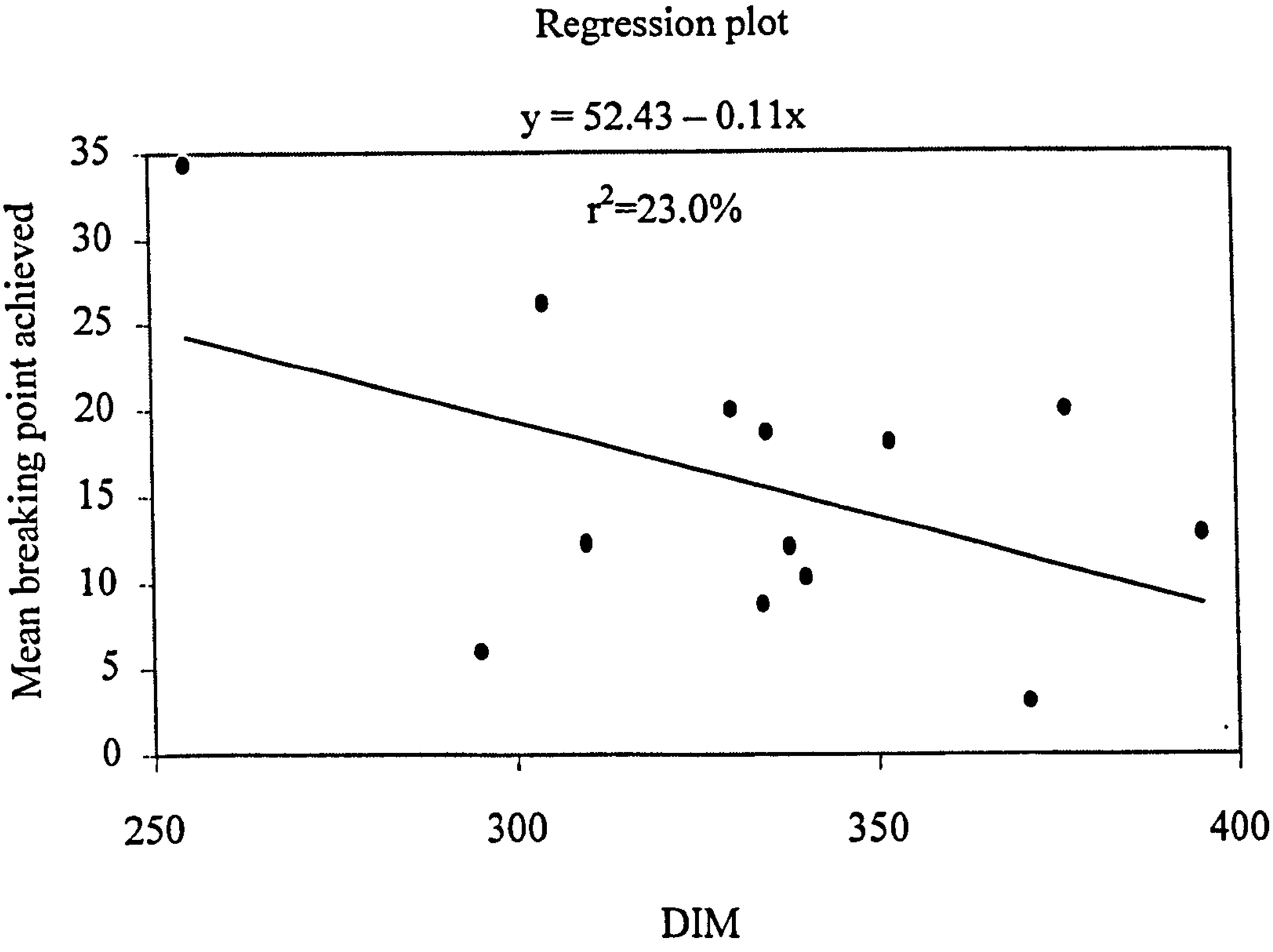


Fig. 4.1 The effect of days in milk (DIM) on the mean breaking points achieved for the experimental animals selected for high and low milk yields

4.4.3 Amount of time spent in the test pen

The GLM detected no differences in the amount of time spent in the test pen between replications for each yield group (P=0.72). There was no significant difference between yield groups in the amount of time spent in the test pen (Table 4.5). There was no significant correlation between time in pen and milk yield. An analysis of covariance indicated that the covariates DIM, BCS and DTP had no effect on the time spent in the pen (P=0.49; P=0.97; P=0.64, respectively). The use of these covariates did not influence the effect of milk yield on the amount of time spent in the pen (P=0.31). DIM, BCS, and DTP were not correlated with milk yield (P=0.39; P=0.46; P=0.71, respectively).

For the high yielding group there was a positive correlation between the amount of time spent in the test pen and the breaking point attained (r^2=71.9, P=0.02). There was no correlation between these variables for the low yielding group (P=0.28).

4.4.4 Mean breaking points achieved per day – by yield group

There was a tendency for the low yielding group to achieve a higher mean breaking point per day for the first nine test days in comparison to the high yielding group (P=0.08) (Table 4.6). The low yielding cows achieved significantly higher breaking points for the first five days, but there was no difference between the two yield groups for days six to nine (P=0.82).

Table 4.6 Mean breaking points (BP) achieved per day for the experimental animals selected for high and low milk yields

Test day	Mean BP		SED ±	P-value	Correlation coefficient		r^2 value		Correlation with test day P-value	
	Lows	Highs			Lows	Highs	Lows	Highs	Lows	Highs
1 to 9	17	14	1.5	0.08	0.516	0.794	-	63.0	0.16	0.01
1 to 5	16	11	0.9	<0.01	0.464	-0.404	-	-	0.43	0.50
6 to 9	17	17	2.6	0.82	0.809	0.978	-	95.6	0.19	0.02

For both yield groups, the mean breaking point achieved each day was plotted against the first nine test days (Fig 4.2). There was a positive correlation between the mean breaking point achieved each day and the first nine test days, for the high yielding group (r^2=63.0, P=0.01) (Fig. 4.3). No correlation existed for the low yielding group (P=0.16).

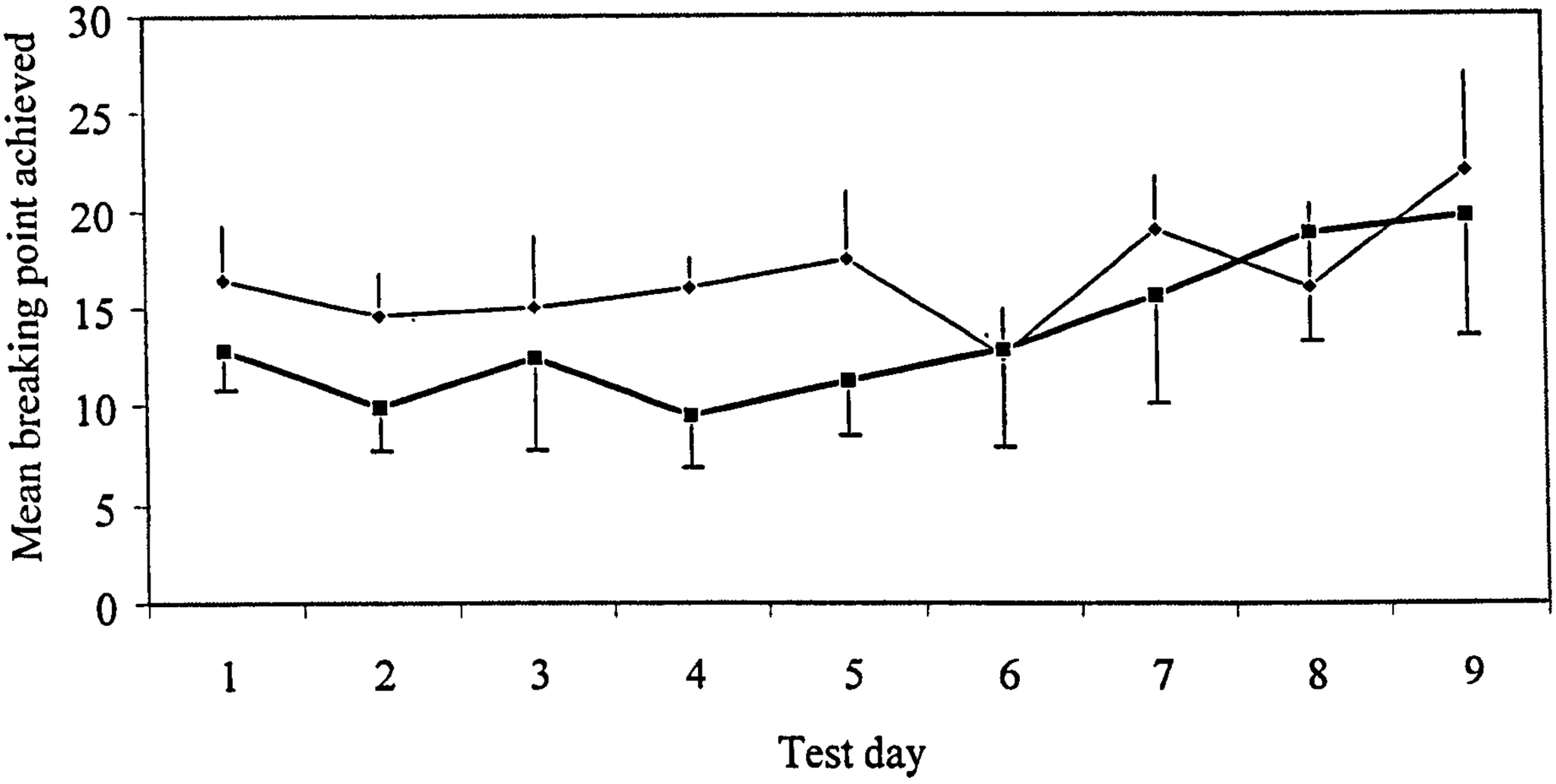


Fig. 4.2 Mean breaking points achieved each day for the experimental animals in the high (■ – ■) and low (♦ – ♦) yielding groups (Plus and minus error bars have been displayed for the low and high yielding groups, respectively) (Plus/minus error bars represent 0.5 S.E.M)

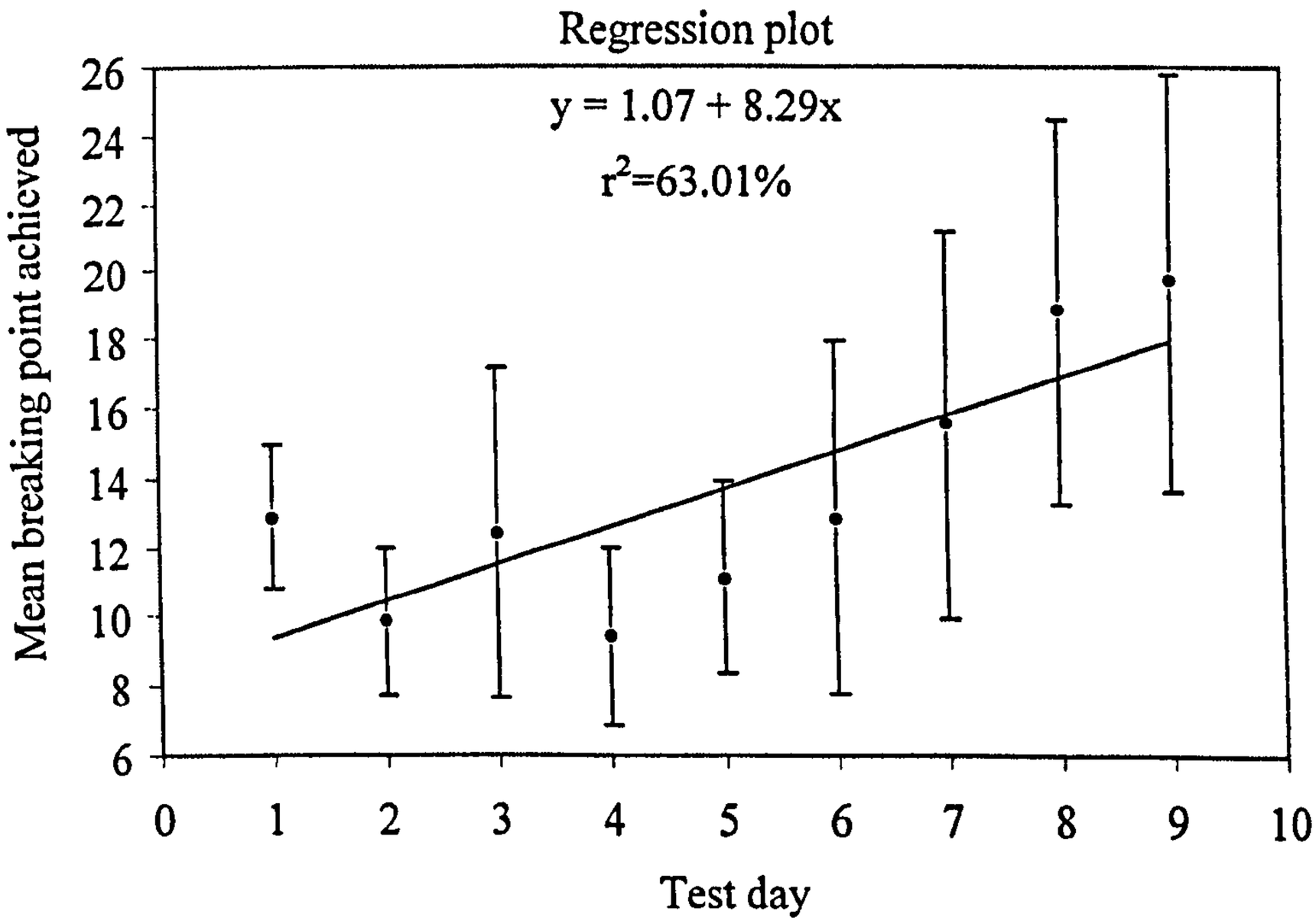


Fig. 4.3 Linear regression plot showing the mean breaking points achieved each day for the high yielding group (Error bars represent S.E.M)

Fig. 4.2 shows that the breaking points increased significantly over the experimental period for the high yielding group. For the low and high yielding groups there was no significant positive correlation between the mean breaking points achieved for days one to five (Table 4.6). However, between days six and nine there was a positive correlation between these variables for the high yielding group only (r^2=95.6, P=0.02). This further demonstrates that breaking points continued to rise towards the end of the experimental period for the high yielding group.

4.4.4.1 Mean breaking points achieved per day – for individual cows

There were significant positive correlations between the breaking points achieved per day and the number of test days for one low yielding cow and two high yielding cows (Table 4.7). Breaking points, for these cows, therefore continued to increase each day for the duration of the test period. One high yielder (cow 95) had a significant negative correlation between these variables. However, a visual inspection of the data revealed that this result may be misleading. This cow consistently obtained a breaking point of three, for 10 of the test days. Unusual breaking points of six and one were observed for days one and 11, respectively. When these observations were removed sequentially, the data was not significantly correlated (P=0.22; P=0.13, respectively). The original correlation is thus not believed to represent a true picture of this cow's progress over the test period. There were no significant correlations between these variables for any other cow.

Table 4.7 Linear regression analysis results between the breaking point attained by each cow per day and the number of test days

Cow number	Yield group	Test group	P-value	Correlation coefficient	r^2 value
47	Low	1	0.01	0.708	50.1
564	Low	1	0.70	-0.125	-
93	Low	1	0.09	0.508	-
51	Low	2	0.07	0.630	-
561	Low	2	0.17	0.499	-
74	Low	1	0.10	0.493	-
112	High	2	0.01	0.841	70.7
21	High	1	0.01	0.706	49.8
95	High	1	0.04	-0.591	-
549	High	1	0.40	0.271	-
138	High	1	0.24	-0.370	-
19	High	2	0.60	0.203	-
52	High	2	0.25	0.430	-

4.4.5 Mean time taken to receive a reward

The GLM indicated no differences between replications in the mean time taken to receive a reward for each yield group (P=0.67). There was no significant difference between yield groups in the mean time taken to receive a reward (Table 4.8).

Table 4.8 Mean time taken to receive a reward for the experimental animals selected for high and low milk yields

Observation	Mean		SED ±	P-value	Correlation coefficient	Correlation with milk yield P-value
	Lows	Highs				
Time taken to receive a reward (seconds)	128	123	23.2	0.84	-0.133	0.67

There was no correlation between this measure and milk yield. BCS, DIM and DTP had no effect on the mean time taken to receive a reward when used as covariates in an ANCOVA (P=0.72; P=0.70; P=0.92, respectively). The use of these covariates did not improve the effect of milk yield on the mean time taken to receive a reward (P=0.80). BCS, DIM and DTP were not correlated with the time taken to receive a reward (P=0.83; P=0.83; P=0.86, respectively).

4.4.6 Mean time taken to receive first and fourth rewards

There were no differences in the mean time taken to receive the first or fourth rewards between replications for each yield group (P=0.49; P=0.57, respectively). There was also no significant difference between yield groups in the mean time taken to receive the first or fourth rewards (Table 4.9).

Table 4.9 Mean time taken to receive the 1st and 4th rewards for the experimental animals selected for high and low milk yields

Observation	Mean		SED ±	P-value	Correlation coefficient	Correlation with milk yield P-value
	Lows	Highs				
*Time to receive 1st reward	38	23	18.7	0.41	-0.226	0.46
*Time to receive 4th reward	349	390	66.1	0.55	0.043	0.90

**Time in seconds*

There was no correlation between time taken to receive the first or fourth reward and milk yield. When an ANCOVA was conducted, using BCS, DIM and DTP as covariates, it did not improve the significance of milk yield on the time taken to receive the first or fourth reward (P=0.51; P=0.36, respectively). BCS, DIM and DTP did not affect the time taken to receive the first (P=0.74; P=0.69; P=0.63, respectively) or fourth (P=0.80; P=0.57; P=0.51, respectively) reward.

4.4.7 Mean time taken to receive each successive reward and intake rate

There was no correlation between the mean time taken to receive successive rewards and the mean number of rewards received for the high or low yielding groups (P=0.45; P=0.28, respectively). Four low yielding and two high yielding cows demonstrated a significant increase in the mean time taken to receive successive rewards as the test session progressed (Table 4.10). Breaking points have been included in Table 4.10 to demonstrate that the increasingly longer time taken to receive each successive reward was not necessarily associated with the achievement of a greater breaking point.

There was no difference between the high and low yielding groups in the mean intake rate of the concentrate reward (205 versus 177 g min^{-1}, respectively. P=0.46). There was no correlation between the rate of intake and milk yield (P=0.56). Using the coefficient values from the regression equation there was no difference between the yield groups for the mean time taken to receive each successive reward (P=0.34).

Table 4.10 Regression analysis results between the mean time taken to receive each successive reward and the number of rewards received

Cow number	Yield group	Test group	P-value	Correlation coefficient	r^2 value	Mean BP* achieved
93	Low	1	<0.01	0.843	71.0	18
47	Low	1	0.02	0.663	43.9	19
74	Low	1	0.02	0.877	77.0	13
51	Low	2	0.03	0.611	37.4	20
561	Low	2	0.47	0.219	-	26
564	Low	1	0.29	0.594	-	10
112	High	2	<0.01	0.741	55.0	34
549	High	1	<0.01	0.943	87.3	12
95	High	1	0.92	0.129	-	3
21	High	1	0.20	0.377	-	20
138	High	1	0.18	0.636	-	12
19	High	2	0.15	0.849	-	6
52	High	2	0.09	0.742	-	9

*BP = Breaking Point

4.4.8 Behavioural observations post-testing

A GLM analysis did not indicate any differences in feeding, drinking or standing behaviours between replications for each yield group (P=0.43; P=0.34; P=0.33, respectively). The high yielding group spent significantly more time feeding and less time standing (Table 4.11). Time spent feeding was significantly positively correlated with milk yield. There was no correlation between time spent standing and milk yield.

There was no difference between yield groups in the amount of time spent drinking or lying (Table 4.11). Neither time spent drinking or lying were significantly correlated with milk yield. DIM, BCS and DTP had no significant effect on any of the

behaviours examined when used as covariates in an ANCOVA. Only feeding time was significantly correlated with DIM (r^2=33.4, P=0.04).

Table 4.11 Mean time spent in each behaviour and the significance level between treatment groups, and the significance value, correlation coefficient and r^2 value for each behaviour when correlated with milk yield

Behaviour	Mean time (min)		SED ±	P-value	Correlation coefficient	r^2 value	Correlation with milk yield P-value
	Lows	Highs					
Feeding	9	17	2.6	0.01	0.656	43.0	0.02
Standing	4	1	0.7	<0.01	-0.392	-	0.19
Drinking	2	1	1.3	0.38	-0.087	-	0.78
Lying	7	2	3.9	0.23	-0.621	-	0.10

4.5 Discussion

The similar breaking points achieved by both yield groups would indicate that the high yielding cows did not appear to have a greater appetite for concentrates. This can also be concluded from the results obtained for the other parameters, *i.e.* the mean time taken to receive a reward, and the mean time taken to receive the first and fourth rewards. These latter measures were taken as possible indicators of eagerness to feed and may have therefore revealed increased levels of motivation. There was also no difference between the yield groups in the amount of time taken to receive each successive reward. However, it was evident from the behavioural observations post-testing that the high yielding cows had a greater appetite for the total mixed ration. The high yielding group spent nearly twice as long feeding on the forage ration post-testing in comparison to the low yielding cows (P=0.01). There was also a significant relationship between the time spent feeding and milk yield. An examination of individual progressive work rates also provided evidence of increased feeding motivation. Five high yielding cows (71% of the group) showed no significant

increase in the time taken to obtain each additional reward. This suggests that these cows were working progressively harder, *i.e.* eating faster and/or pressing quicker, as further rewards were acquired. If the cows were responding at a constant rate, it would take them progressively longer to complete the increasing series of lever presses to obtain each successive reward. This result may therefore indicate an increased motivation to feed in these cows. In contrast, only two low yielding cows (33% of the group) showed a similar response, but there was no overall difference in the mean time taken to receive each successive reward between the two yield groups. It was not the case that those cows taking progressively longer to obtain additional rewards were achieving higher breaking points. Likewise, a correlation analysis revealed a strong relationship between the time spent in the test pen and the breaking points attained for the high yielding group (P=0.02). Thus, although the high yielding cows spent a similar amount of time in the test pen as the low yielding cows, this time was dedicated more to working for food rather than spent as non-responding time. This would imply that the high yielding cows were making better use of their time whilst in the pen. There was a tendency for the mean breaking point achieved to decrease as the number of days in milk increased. This was expected as dry matter intake declines in late lactation (Phillips, 2000b).

There are several possible reasons why there was no significant difference in breaking points between the yield groups. It was initially thought that the high yielding cows were following an optimal foraging theory (OFT). An animal selects food not only according to its palatability and quality (Baumont, 1996) but also in relation to its cost (Pyke, 1984; Stephens and Krebs, 1986). The OFT suggests that the costs associated with obtaining a food will influence its rate of intake (Pyke, 1984). According to the

theory animals aim to maximise their net rate of energy gain. If the energy values of the concentrate and the total mixed ration are examined, the difference in their predicted energy values was small (12.4 versus 12.2 MJ kg DM, respectively), and it was possible that the cows believed they were better able to increase their energy intake per unit time by feeding on the forage-mixture. However, the high and low yielding cows were feeding on the concentrate at mean intake rates of 178 and 153 g DM min^{-1}, respectively, which is far greater than those rates achieved on forage (50-60 g DM min^{-1}, Phillips, 2002). Even towards the end of each test, no cow reduced its concentrate intake rate to that expected for feeding on forage (mean lowest intake rate for all cows was 99 g DM min^{-1}; range = 77–163 g DM min^{-1}).

It was also considered possible that the cows stopped working at a fixed point due to the effect of the concentrate reward on the rumen environment. High amounts of concentrate cause propiogenic rumen bacteria to proliferate, which cause the pH to fall, reducing fibre digestion (Blowey, 1985). This usually results in a loss of appetite and cattle will stop feeding for up to 2 h after a large concentrate intake (Blowey, 1985). Webster (1993a) stated that to prevent this condition cows should be restricted to 4 kg of concentrate per feed. The high and low yielding cows received a total mean concentrate reward of 1.7 and 2.2 kg, respectively, per test session, which is well below this suggested level. Even when looking at individual cow's mean reward values only one cow (no. 112) received a total reward value near this amount (3.9 kg). Additionally, the cows continued to feed on the forage-mixture available post-testing, which demonstrates that their appetites were not suppressed.

Another reason for the non-significant difference in breaking points could be considered as a learning issue. Initially, the low yielding group obtained higher breaking points per day for the first five days, in comparison to the high yielding group, but from day six onwards this difference was not observed. There was a relationship between the breaking points achieved and the number of test days for the high yielding group. In other words, the high yielding cows were continuing to increase the number of lever presses achieved each day, which means they may have not reached their peak breaking point potential as a group. This increase in breaking points was only evident after an initial refractory period between days one and four. However, an examination of individual cow effort revealed that this result was primarily due to only two of the high yielding cows, which were observed to progressively increase their breaking points each day. Conversely, only one low yielding cow performed this. This highlights the importance of considering individual variability when interpreting such results (Nicol, 1986).

A further consideration is that the test itself was not appropriate and failed to demonstrate the purpose for which it was designed, *i.e.* the lever pressing device may not have enabled the cows to express, in a way natural to themselves, their level of feeding motivation (Dawkins & Beardsley, 1986). Fraser and Mathews (1997) stated that such tests should be set within the limits of the sensory and cognitive capacity of the animal. For example, Jackson *et al.* (1999) found that when sheep were required to press a panel for food, motivation to obtain more food did not increase with increasing food deprivation. When they used an alternative method, which required the sheep to run down a race and enter the reward via a weighted door, their motivation was shown to significantly increase with food deprivation. Although this is

an important consideration, it is unlikely to have been significant in this case as similar studies with cows have shown that they can successfully use lever pressing devices to make informed choices (Mathews & Temple, 1979; Klopfer *et al.* 1981; Arave *et al.,* 1983). Nevertheless, it would be useful to test the cows' motivation to feed using an alternative operant procedure.

The most likely reasons for their being no difference in breaking points are twofold. There may be an absolute time limit that cows are prepared to work for a food reward in the knowledge that there is an alternative food source available that is 'free'. The cows may have learnt that the forage ration is available to consume after the test, which reduced their motivation to work for additional concentrate rewards. Hursh *et al.* (1988) demonstrated that food-deprived rats are less likely to work as hard for a food reward if they know they will receive food at the end of the experimental period. It could also be that there came a point where the cows felt that they had spent long enough working for the concentrate, which may have been dictated by the frustration experienced by obtaining further rewards after they had reached a certain level. Time spent feeding could be considered preferable to time spent working for food, even though the concentrate reward can be consumed at a greater rate once achieved. This may be especially the case for the high yielding cows. Their increased feeding time shows that it takes longer to satisfy their appetite. The extra time spent having to work for food may mean that less time is available to perform other desirable activities such as rest. The cows may have worked for as long as they did to vary their diet, or the test itself may have acted as a form of enrichment.

In conclusion, under the conditions of this experiment, the high yielding dairy cows did not demonstrate, with reference to the breaking points achieved, a greater appetite for concentrate. However, they spent significantly longer feeding post-testing on the total mixed ration provided. It is presumed that the cows worked to a point where they considered that any continued effort would have been an insufficient use of time, and thus the 'free' forage available post-testing was a preferable option. This may have been determined by the onset of a negative psychological experience, such as frustration, caused by the increasing number of presses required to obtain successive rewards after reaching a certain level. Further research could test this assumption by removing the possibility to feed after the experiment, reducing the PR interval and thus potentially allowing more frequent rewards in the same time period, or by offering rewards on a varying time schedule. Progressive work rate may have provided a better indication of feeding motivation for individual cows. It may also have been beneficial to run the experiment over a longer time period until the breaking points for the high yielding group peaked. Alternative tests could also be used to investigate feeding motivation.

5a. The effect of short-term lying deprivation on the behaviour of lactating dairy cows

5.1a Introduction

The opportunity to satisfy the motivation to lie down is important to ensure the well-being of dairy cows (Metz, 1985; Wierenga & Hopster, 1990; Munksgaard *et al.*, 1999). Studies have shown that cattle demonstrate a strong motivation to carry out this behaviour (Metz & Wierenga, 1984; Metz, 1985; Wierenga & Hopster, 1990; Hopster *et al.*, 2002; Jensen *et al.*, 2002) and its prevention, even for a short period, can be considered stressful and aversive (Munksgaard & Simonsen, 1996; Fisher *et al.*, 2002). Lying allows the animal to rest and sleep, which are important for brain health and the recovery of body systems (Fraser & Broom, 1997). The amount of time dairy cows will spend lying will vary according to a number of factors, such as their age (Phillips, 1993), physiological state (Chaplin & Munksgaard, 2001) and the management system in which they are kept (Wierenga & Hopster, 1990; Fisher *et al.*, 2003).

Both physical and physiological factors can compromise the amount of time available for lying. A previous experiment (Chapter 3b) revealed that milk yield had a significant effect on lying time: As milk yield increased the time spent lying decreased. This was probably due to the higher yielding cows requiring a longer grazing period and, as a consequence, had less time available to lie. Atkeson *et al.* (1942) and Castle and Halley (1953) have also observed this negative relationship with grazing cows. Similarly, Fregonesi and Leaver (2001) observed that high yielding cows had shorter lying times but longer feeding times whilst indoors, compared to low yielding cows. This association may not be so surprising considering

the time devoted to these activities may occupy up to eight hours per day each (Webster, 1995), leaving little else to give up in order to continue feeding. In addition, cows in early lactation have been found to spend less time lying than cows in late lactation and dry cows (Veris *et al.*, 1980; Chaplin & Munksgaard, 2001). This may also be a milk yield effect, as yields tend to be greater during the early stages of lactation. The housing conditions in which dairy cows are kept can also affect lying behaviour. For example, the lying time of cows in cubicles has been shown to reduce in proportion to the degree of overcrowding (Wierenga, 1983; Wierenga & Hopster, 1990). This has been observed to occur mainly among lower-ranking individuals during periods of mass rest, *i.e.* at night (Friend *et al.*, 1977). Similarly, a reduced space allowance, simulating that experienced during transportation, has been shown to increase standing time and reduce lying time in red deer (Grigor *et al.*, 1997). The housing system itself can also affect lying times. Cows in strawyard systems have been observed to lie for longer than those in cubicles (Phillips & Schofield, 1994; Fregonesi & Leaver, 2001). Also, routine farm procedures, such as artificial insemination, can force cows to spend long periods of time standing, possibly up to four hours, whilst waiting for a practitioner to come and carry out the procedure (Phillips, 2001). In addition, in some countries, such as New Zealand, farmers remove cows from pasture to prevent pasture damage during periods of heavy rainfall. Throughout such periods, cattle can be confined to concrete yards, where they have been observed standing for long periods, as they were deterred from lying on the hard surface (Fisher *et al.*, 2003). Fisher *et al.* (2003) also reported that, under such conditions, these 'stand-off' periods can result in sub-optimal daily lying times for cows.

Lying is considered to be such an important behaviour that it can be used as an indicator to assess dairy cattle welfare (Miller & Wood-Gush, 1991; Krohn *et al.*, 1992; Fregonesi & Leaver, 2001). Lying deprivation can have negative effects on both the health and welfare of dairy cows. Munksgaard and Simonsen (1996) found that cows repeatedly deprived of lying had greater ACTH concentrations at the beginning and end of a 14 h deprivation period, which indicates that the procedure is stressful and that the animal is unable to adapt to the stress within this period. This has also been confirmed by Munksgaard *et al.* (1999) and Fisher *et al.* (2002) who found that repeated lying deprivation increased the cortisol response to an ACTH challenge test. Increased levels of stress, as a direct result of forced standing, can be responsible for lowering immune system function and hence decrease resistance to disease (Fisher *et al.*, 1999). Increased stress can also affect the production of the growth hormone somatotrophin. Lower levels of this hormone have been found in calves housed in spatially restricted crates that prevented them from adopting normal resting positions, in comparison to calves kept in pens (de Passille & Rushen, 1995). Correspondingly, Munksgaard and Lovendahl (1993) and Ingvartsen *et al.* (1999) recorded lower levels of growth hormone in cattle that were deprived of lying for 14 h per day for several weeks. As growth hormone has a positive effect on lactation performance (Munksgaard & Lovendahl, 1993) a decrease in its production could result in a reduction in milk yield (Hart *et al.*, 1978). The prevention of lying can also be responsible for predisposing cows to other health problems such as sole haemorrhages (Singh *et al.*, 1993a). All of these factors mentioned not only impact on the welfare of the animal, but also on the economic viability of the farmer (Galindo *et al.*, 2000). Within the scientific community, depriving cows of lying has been considered such a stressor that it has been used to determine the effects of stress on

pituitary-adrenal axis activity and regulation (Munksgaard & Simonsen, 1996; Fisher *et al.*, 2002), growth hormone levels (Munksgaard & Lovendahl, 1993) and production parameters (Verkerk *et al.*, 1999).

Deprivation experiments offer a particularly useful methodology to assess an animal's need to perform a specific behavioural activity. The procedure requires that the animal be prevented from carrying out a specific behaviour for a period of time. There are behaviours that are prompted by external cues and would be expected to occur at the same rate after a period of deprivation (Kennedy, 1985). However, there are some behaviours that are activated by internal drives, which become stronger as the time since they were last performed increases (Jensen, 1999). This build up of motivation is usually expressed by an increase in the rate or intensity of the behaviour after a period of deprivation (Petherick & Rushen, 1997), and is termed a rebound effect. Behaviours that show this rebound effect are thought to have some important function, can be regarded as behavioural needs and are likely to cause some level of frustration and possibly suffering if prevented (Dawkins, 1988; Webster, 1995; Petherick & Rushen, 1997). The greater the rate of recovery, the more important the behaviour is to the animal, and the more the welfare of the animal will be compromised if it is prevented from performing it. Examples of such behaviours include crib-biting and social interactions in horses (McGreevy & Nicol, 1998; Christensen *et al.*, 2002), wing-flapping and stretching in battery hens (Nicol, 1987) *and locomotor behaviour in calves and heifers (Jensen, 1999). During a period of* denial, an animal may also show some displacement activity. These are purposeful behaviours but are not relevant to the current situation the animal is facing. It is thought that an animal will perform these to help overcome the negative feelings a

situation is imposing. For example, Munksgaard and Simonsen (1996) suggest that eating, idling and grooming are possible displacement activities in dairy cows during lying deprivation. Displacement behaviours can therefore be recorded to indicate the level of frustration an animal is experiencing (Munksgaard and Simonsen, 1996).

The aim of the current study was to determine whether a short-term reduction in lying time compromises the welfare of lactating dairy cows. The study was particularly interested in understanding the possible stress experienced during lying deprivation by high yielding cows when grazing. Behavioural observations were employed to reveal any displacement or rebound activities. Pre- and post-deprivation milk yields were also recorded as an indication of possible production changes.

5.2a Materials and methods

5.2.1a Animals

An experiment was conducted over 20 consecutive days, between 13th May and 1st June 2002 inclusive, using cows from the Holstein-Friesian dairy herd of Moulton College, Northampton. The management system employed dictated that the herd of 189 dairy cows was divided into three lactating groups according to stage of lactation: High yielders (cows at peak lactation), mid yielders, and low yielders (cows nearing end of lactation). The low yielding group, which consisted of 80 dairy cows, was selected for this study. The experiment was conducted with ten groups (blocks) of six cows. Cows within each block were paired and assigned to one of three treatments: Control (Treatment C) (no lying deprivation), 2 h of lying deprivation (Treatment 2), and 4 h of lying deprivation (Treatment 4). There was no difference between treatments C, 2 and 4 in initial milk yield (24.8 kg d^{-1} (s.e. 1.43), 25.0 kg d^{-1} (s.e. 1.40), 22.5 kg d^{-1} (s.e. 1.17), respectively. P=0.22), based on National Milk Records (NMR) taken on 15th May 2002 (Table 5.1a). Treatments C and 2 contained animals of a similar age. The animals used in treatment 4 were approximately 12 months younger than those used in the other two treatments (P=0.04) (Table 5.2a). All cows were scored for body condition by the same assessor on a scale of one to five, to within 0.25 of a unit (Edmonson *et al.*, 1989), on three separate occasions during the experiment (Table 5.1a). There was no difference between the treatments in mean body condition score (P=0.576), days in milk (P=0.09), or days to parturition (P=0.08) (Table 5.2a). Data on days to parturition were not available for some cows (Table 5.1a).

Table 5.1a Characteristics (cow no., block no., mean body condition score (BCS), lactation number, days in milk, days to parturition, and milk yield) of the experimental animals selected for the control (a), 2 h (b) and 4 h (c) treatments (*data not available)

(a) Control treatment

Cow Number	Block Number	Mean BCS	Lactation Number	Days in Milk	Days to Parturition	Milk Yield (kg d^{-1})
22	1	3.00	3	232	122	23.8
3	1	3.38	5	216	196	23.4
26	2	3.13	2	889	*	16.0
307	2	2.88	3	323	*	7.2
11	3	2.63	3	213	161	35.8
173	3	2.25	2	250	127	28.4
90	4	3.00	5	240	*	17.8
136	4	2.50	1	216	148	25.4
118	5	3.13	5	289	149	21.2
185	5	3.13	5	244	115	21.2
34	6	2.63	1	288	76	24.4
181	6	2.75	3	227	181	27.8
166	7	2.38	4	209	*	30.2
220	7	2.88	1	621	*	25.6
54	8	3.25	1	254	*	20.8
114	8	3.00	4	271	*	28.2
117	9	2.88	1	265	112	30.2
49	9	3.13	4	311	*	31.2
188	10	2.63	1	109	*	28.4
105	10	2.63	1	147	186	29.8

(b) 2h deprivation treatment

Cow Number	Block Number	Mean BCS	Lactation Number	Days in Milk	Days to Parturition	Milk Yield (kg d^{-1})
55	1	2.88	1	206	*	28.6
177	1	2.50	2	255	102	25.6
42	2	4.38	3	186	158	19.0
14	2	4.38	4	268	*	7.8
509	3	2.25	6	233	126	31.0
104	3	2.75	1	226	132	31.8
69	4	2.75	3	170	*	27.8
587	4	2.75	2	256	210	29.2
585	5	3.25	2	268	98	14.8
233	5	2.50	2	260	69	21.6
171	6	2.38	1	109	*	24.5
532	6	3.13	1	609	*	16.2
161	7	3.00	1	246	121	25.6
563	7	2.88	5	200	*	28.4
76	8	2.75	3	272	77	24.0
2	8	3.00	3	243	116	26.8
552	9	3.50	3	263	105	31.6
542	9	3.50	4	610	*	26.6
566	10	3.13	4	271	*	29.0
108	10	2.63	4	184	*	30.0

(c) 4h deprivation treatment

Cow Number	Block Number	Mean BCS	Lactation Number	Days in Milk	Days to Parturition	Milk Yield (kg d^{-1})
139	1	3.00	1	249	132	27.8
73	1	3.13	2	948	*	11.4
9	2	3.13	1	528	160	20.0
92	2	3.00	3	325	*	20.4
110	3	3.25	5	260	*	20.4
98	3	2.75	2	525	*	23.0
149	4	2.63	1	213	118	28.4
132	4	3.00	1	247	110	31.4
304	5	3.00	2	553	*	20.0
261	5	3.50	2	301	97	16.4
590	6	2.88	2	214	131	26.4
120	6	2.88	1	267	70	18.4
125	7	2.25	3	318	*	26.4
284	7	2.75	2	307	115	21.0
115	8	3.00	5	268	87	24.2
30	8	2.88	2	286	*	14.0
148	9	2.75	1	242	*	27.8
134	9	3.13	1	263	117	19.6
135	10	2.50	1	261	79	26.2
145	10	2.25	2	217	119	27.0

Table 5.2a Details of average values and ranges for milk yield, body condition score (BCS), lactation number, days in milk (DIM), and days to parturition (DTP) for the experimental animals in the control (C), 2 h (2) and 4 h (4) treatments

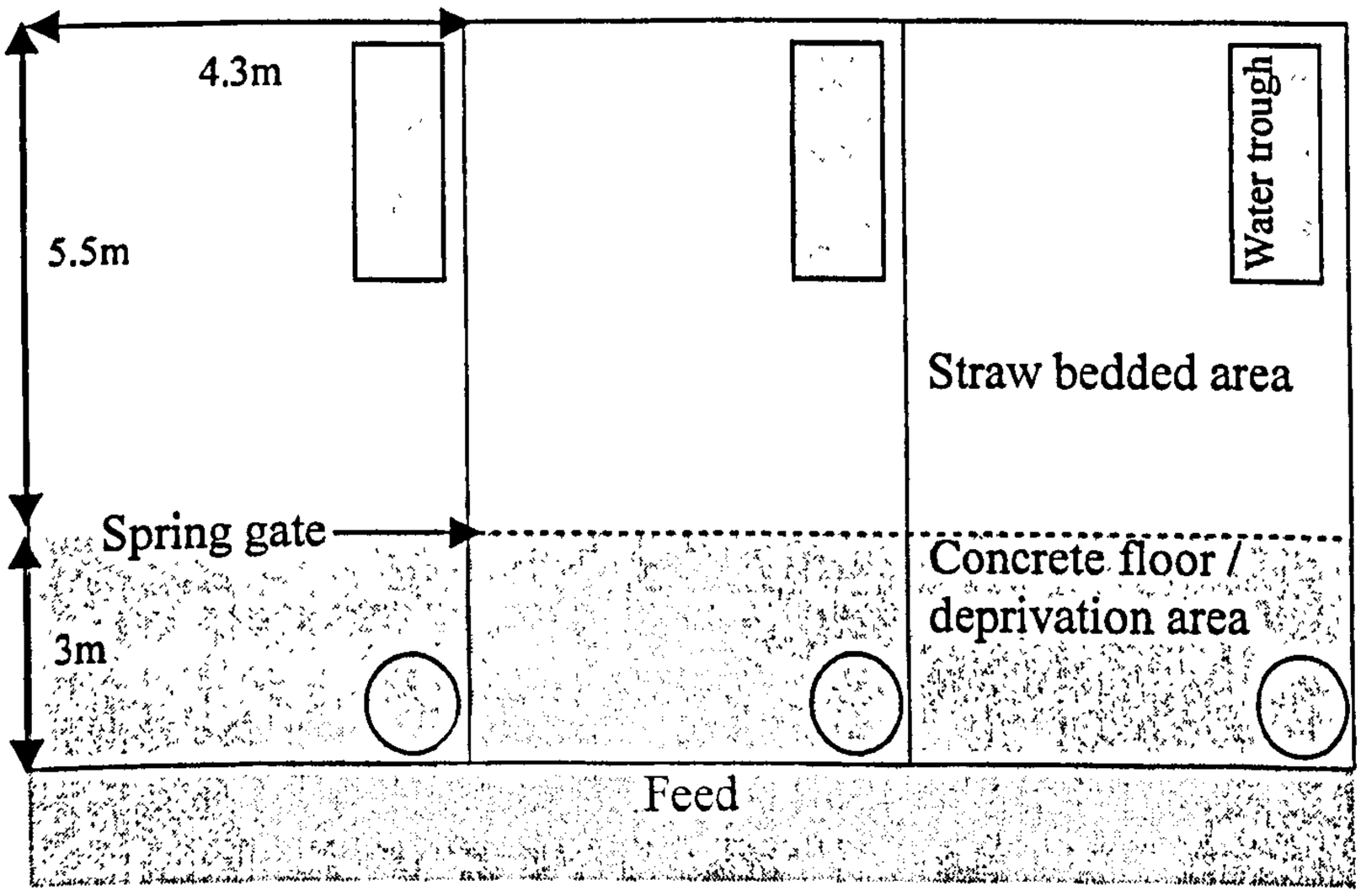
Measure	Group	Mean / *Median	SED ±	Range	P-value
Milk Yield (kg d^{-1})	C	24.8	2.30	7.2 – 35.8	0.22
	2	25.0		7.8 – 31.8	
	4	22.5		11.4 – 31.4	
BCS	C	2.86	0.160	2.25 – 3.38	0.576
	2	3.02		2.25 – 4.38	
	4	2.88		2.25 – 3.50	
Lactation number	C	3*	-	1 – 5	0.04
	2	3*		1 – 6	
	4	2*		1 – 5	
DIM	C	247*	-	109 – 889	0.09
	2	251*		109 – 610	
	4	268*		213 – 948	
DTP	C	143	17.7	76 – 196	0.08
	2	119		69 – 210	
	4	111		70 – 160	

5.2.2a Experimental conditions

When the cows were not undergoing the experiment they were kept together as part of the herd of 80 cows and grazed in a 4.74 ha paddock between 09.00 and 05.00 h with *ad libitum* access to drinking water and an Italian ryegrass pasture (*Lolium multiflorum*) (sown 1999, composed of equal mixes of: Atalja IRG, Ligrande IRG, Solid Hybred Tetraploid IRG, and Donergo Tetraploid IRG), which was kept at the recommended height of 8-10 cm (Chamberlain & Wilkinson, 1996). From 05.00 to 09.00 h, the cows were housed indoors in a large, naturally lit, straw bedded building (70 x 15 m) with access to drinking water and a total mixed ration [maize silage (41.1%), second cut grass silage (27.4%), concentrate pellets (Moulton balancer, Heygates Ltd, Northampton, UK) (16.6%), wet maize gluten (Trafford gold, Heygates Ltd, Northampton, UK) (7.8%), molassed sugar beet (3.9%), lucerne pellets (2.9%) and minerals (0.3%)]. Feed was delivered once per day during morning milking using a Keenan mixer wagon. Chopped straw was provided daily, in appropriate amounts to keep the bed surfaces clean. During periods of darkness, artificial lighting was provided at either end of the shed. All cows were milked twice daily, from c. 05.30 to 07.00 h, and c. 16.00 to 17.30 h. A concentrate allowance of 1 kg (Grassmaster 18, Heygates Ltd, Northampton, UK) was offered in the parlour to each cow per milking.

During the experiment, the cows were housed in pairs in adjacent pens (8.5 x 4.3 x 4.2 m) (L x W x H) with *ad libitum* access to drinking water and a standard total mixed ration (as above) (Fig 5.1a). The pens were situated within a larger barn with housed cows. The pens were constructed from Poldenvale hurdles so that the cows had visual, auditory, olfactory and some minimal physical contact with one another, and did not restrict the vision of the observer monitoring the cows' behaviour. Feed was delivered

once per day during morning milking using a Keenan mixer wagon. During periods of darkness, artificial lighting was provided overhead by six fluorescent tube lights to facilitate video recording of behaviour. All cows were milked twice daily, between c. 06.20 and 08.35 h, and c. 15.10 and 17.00 h, and milking lasted a mean time of 48 and 51 min per block of cows, respectively. A concentrate allowance of 1 kg (Grassmaster 18, Heygates Ltd, Northampton, UK) was offered in the parlour to each cow per milking. Faeces were removed manually from the bedded and concrete areas twice daily during milking times. Clean straw was also provided daily, in appropriate amounts to keep the bed surfaces clean. All cows were in good health at the start of the study, in particular they had no obvious signs of mastitis or clinical lameness.



Circles indicate water bowls available to deprived cows during deprivation period

Fig 5.1a Plan of experimental pens

5.2.3a Experimental procedure

As cows are social animals, the possibility exists that the behaviour of an individual may be dictated by that of the herd. For example, Rook and Huckle (1995) believe

that the grazing behaviour of dairy cows may be socially facilitated, with the commencement and duration of this period being dependent on a few individuals within the group. Various researchers have also reported on the synchronisation of other behaviours, such as lying (Atkeson *et al.,* 1942; Schmisseur *et al.,* 1966; O'Connell *et al.,* 1987). However, although this behaviour may be synchronous, it is not thought to necessarily affect total lying time, but more the commencement of lying (Wierenga & Hopster, 1990). The experimenters therefore attempted to cater for the possibility of such interdependence by housing the subjects in pairs and for a period of only 48 h. If the cows were housed singly, this may have affected their welfare and consequently their behaviour (Munksgaard & Simonsen, 1996; Watts & Stookey, 2000), and a larger group size may have succumbed to the effects of allelomimicry. If the cows had been housed together for a longer period, the more dominant cow of the pair may have emerged and started to alter the behaviour of the other cow.

5.2.4a Cow management

Every two days, six cows were selected from the group during morning milking. Cows were selected according to their order of exit out of the parlour, as long as they had not previously been used in the experiment. The six cows were divided into pairs at random and each pair was assigned to its own experimental pen (Fig. 5.1a). The experimental pen in which the cows were housed dictated the treatment they underwent. The treatments were assigned to each pen at random (using the random number generator on a calculator) for each block of cows, to avoid confounding effects of pen and treatment. Each block of six cows spent 48 h in the experimental pens and was returned back to the herd during morning milking, when a new block of

six cows was selected. Each block of experimental cows had approximately three hours to become accustomed to the pens and their pen mate before behavioural observations commenced. If the paired cows natural markings or features were too similar that they could not be easily distinguished, one cow of the pair was marked with an 'X' on either side of her body using a black or white marker spray (Ritchey stock marker). Lying deprivation was achieved by securing the cows into the deprivation area using a spring gate (Rutland electric fence 17-120) to prevent access to the bedded area (Fig. 5.1a). It was not necessary to electrify the fence. The four hour deprivation period commenced at 10.20 h and the two hour period started at 12.20 h. Both deprivation periods therefore terminated at 14.20 h, at which time the spring gates were removed and the cows gained access to the bedded area. The relative discomfort of the concrete floor, the presence of a small amount of urine and faeces, and being in a confined area appeared to discourage the cows from lying in the deprivation area. An observer was present throughout the deprivation period to ensure the cows did not lie down. The timing of the deprivation periods were considered most appropriate as a pilot study had revealed that the cows were motivated to lie during these times. In addition, they did not interfere with farm management practices, such as milking schedules. The timing and length of the deprivation periods were also selected to be as practically relevant to the real situation faced by high yielding dairy cows as possible, *i.e.* the subjects were deprived during the day, as this is when high yielding cows spend extended periods standing in order to continue grazing (Chapter 3b). A total mixed ration (as described earlier) and water was available *ad libitum* during the deprivation period.

5.2.5a Milk yield recording

Milk yield was recorded on four consecutive occasions for each cow whilst undergoing the trial to examine any possible effects of lying deprivation on milk production. Records were taken at afternoon milking on day one (the deprivation day) (first milking), morning and afternoon milking on day two (second & third milking), and morning milking on day three (fourth milking), when the cows were returned back to the herd. Records were taken by two observers, who had been trained to record milk yields by the NMR data collector. NMR taken on 15th May were used as control recordings for the cows.

5.2.6a Behavioural observations during deprivation

The behaviour of each cow was recorded during the deprivation period by three observers. A single observer recorded all cows at any one time. Observers were rotated sequentially. All observers were trained in the behavioural recording methods employed to reduce interobserver error. The cows were always observed in the same order.

5.2.6.1a Continuous recordings

The behavioural activities of all animals were recorded using continuous recording (Martin & Bateson, 1995), which measured the frequency and/or duration of each behaviour pattern as it occurred. Bouts were separated by the cow showing at least 1 s of another behaviour. The behaviours were selected to provide possible indicators of frustration or fatigue. A new behavioural recording sheet was used each hour to examine whether certain behaviours changed in frequency over the deprivation period. The behaviours were previously determined by spending one eight hour period

with the cows, which included observing some cows continuously standing for up to two hours during milking times. The 24 mutually exclusive ethological characteristics selected for analysis are represented in Table 5.3a.

Table 5.3a Behaviours recorded continuously by frequency (a) and duration (b) during the lying deprivation period

(a) Behaviours recorded by frequency

Behavioural variable	Definition
Leg raising	Lifting hoof and replacing on same spot without forward momentum (Houpt *et al.*, 2001)
Repositioning	Only performed by deprived cows due to confined area. Moving all four legs slowly forward one at a time in a synchronised manner covering less than one body length in distance.
Walking	Moving all four legs slowly forward one at a time in a synchronised manner covering greater than one body length in distance.
Butting	Use of head by one cow to physically displace the other (Phillips, 2001)
Pushing	Deliberate use of a part of the body, other than the head, to physically displace the other cow (Phillips, 2001)
Threat	Where the head was swung in the direction of the other cow, who took avoidance action (Phillips, 2001)
Vocalising	Cow making sound with mouth either open or closed (Sandem *et al.*, 2002)
Head swing	Swinging of head either upwards or to the side
Feed tossing	Tossing feed into the air with nose
Lying	Body resting on floor (only for deprived cows) (Munksgaard *et al.*, 1999)
Weight shifting	Displacing weight from one side of the body to the other by either relaxing a leg or shuffling the legs
Grooming self	Self licking any part of the body
Grooming other	Licking any part of the body of the other cow
Sniff ground	Sniffing the ground
Nose ground	Nosing the ground
Lick ground	Licking the ground
Lick housing*	Licking the housing

(b) Behaviours recorded by duration

Behavioural variable	Definition
Leaning against housing*	Leaning of body against housing
Rubbing body against other	Rubbing of any part of the body or head against the other cow
Rubbing body against housing*	Rubbing of any part of body, except the head, against housing
Rubbing head against housing*	Rubbing of head against housing
Sniff housing*	Sniffing the housing
Drinking	Drinking from water trough/bowl
Sleeping	Lying down with neck relaxed and eyes closed

*Housing refers to gates, walls, feeding barrier and water trough

5.2.6.2a Instantaneous recordings

The behavioural activities of all animals were also recorded using instantaneous scan sampling (fixed interval time point sampling or point sampling) (Martin & Bateson, 1995) at 5 min intervals. These time intervals have been shown to provide an accurate analysis of the major behavioural activities such as feeding (Hull *et al.*, 1960). The five mutually exclusive ethological characteristics selected for analyses were: Feeding, standing, ruminating standing, ruminating lying and lying (Table 5.4a). For each individual cow the total time spent in each activity was estimated by assuming that they had been performing the behaviour for the remainder of the 5 min period and multiplying the number of recordings of each behaviour by the number of scans in the four hour period.

Table 5.4a Behavioural categories recorded instantaneously during the lying deprivation period and from the videotapes post-deprivation

Behavioural variable	Definition
Feeding	The animal has food in the mouth and/or chews (Munksgaard *et al.*, 1999)
Ruminating lying*	Chewing regurgitated cud whilst lying
Ruminating standing*	Chewing regurgitated cud whilst standing
Standing	Standing (excluding feeding and ruminating)
Lying	Body resting on floor (Munksgaard *et al.*, 1999) (excluding lying ruminating)

*Only recorded during lying deprivation period

5.2.7a Behavioural observations post-deprivation

The behaviour of each cow post-deprivation was quantified by the same observer.

5.2.7.1a Lying position and changes in posture

For one hour after the deprivation period, the lying position (*i.e.* either lateral or sternal) and the number of changes in posture were recorded for each cow that was

observed lying. If a cow was laterally recumbent then her lying side, *i.e.* left or right, was also noted.

5.2.7.2a Post-deprivation video observations

Continuous time-lapse (2.6 mm s^{-1}, *i.e.* one 3 h tape for every 24 h) monochrome video recordings commenced immediately after the deprivation period for the following 41 h spent in the pen for each replicate. A 41 h period was selected as it was anticipated, from the literature, to provide ample time for any behavioural effects of the deprivation to be demonstrated (Metz, 1985). Tapes were changed whilst the cows were being milked in the afternoon. The videotapes were analysed by instantaneous scan sampling (Martin & Bateson, 1995) at 5 min intervals. Observations were conducted during all periods except when the cows were being milked. The following behaviours were recorded: Feeding, standing and lying. Table 5.4a describes the mutually exclusive behavioural categories quantified. For each individual cow the total time spent in each activity was estimated by assuming that they had been performing the behaviour for the remainder of the 5 min period and multiplying the number of recordings of each behaviour by the number of scans in the 41 h period. The videocassette recorder was a VHS Mitsubishi HS-1024EB. The camera was a Sanyo 3372 fitted with a varifocal (3.5 – 8 mm) lens.

5.3a Statistical analyses

All statistical analyses were performed using the 13th release of the Minitab statistical programme (Minitab, 2000). A probability value was considered significant when $P \leqslant 0.05$. A probability value between 0.05 and 0.1 was referred to as a trend or tendency. Individual cows were used as replicates for all statistical analyses (see Chapter 5b for justification of this procedure). Normal distributions were defined by the Anderson-Darling test ($P \geqslant 0.05$), and tests for homogeneity of variance were defined by Bartlett's test if normally distributed, or Levene's if not normal ($P \geqslant 0.05$). The F-Test replaced Bartlett's when there were only two levels for comparison. Where the Kruskall-Wallis test was used, this was always selected over the Mood's Median, as it is considered a more powerful test (the confidence interval is narrower) for analysing population medians from multiple distributions (Minitab, 2000). Where it was selected, the Mann-Whitney test was preferred over the Kruskall-Wallis and the Mood's Median as the former is an alternative to the two-sample t-test and is more robust, less easily influenced by outliers, and is a comparatively stronger test for analysing population medians from two distributions (Minitab, 2000).

5.3.1a Milk yield recordings

Milk yields were tested for treatment differences using the General Linear Model (GLM), providing that the values for each treatment were normally distributed and all treatments to be compared had equal variance. Data not following a normal distribution were normalised using the square of the original data, unless otherwise stated. The GLM was selected to adjust for differences between replicates, as revealed by an analysis of variance (ANOVA) test. As there was a tendency for the pre-experimental milk yields to be different between treatments, the NMR data were used

as a covariate for post-deprivation milk yield analysis. No data were resistant to mathematical transformation to normalisation. All data for all treatment combinations were homogenous. For the pre-experimental milk yield comparisons, the milk yield data for all six cows in block one were not included for analysis as these cows were subject to the experimental conditions before the NMR data were taken. For the NMR recording, all treatments had data following a normal distribution for milk yields taken during the morning and afternoon milkings. When the data for these periods were combined, only data for treatment 2 were not normally distributed.

All data, for each treatment, taken during the first and second milkings, and when combined, and during the third and fourth milkings, and when combined, and when averaged for both recordings, were normally distributed except treatment C during the second milking, the first and second milkings combined, and the average morning milking; treatment 2 during the second milking; and treatment 4 during the fourth milking, and the average morning milking. Logarithms of the original data were used to achieve normal distributions for treatment 4. The average morning milking data for treatments C and 4 each required different methods of transformation to normality, therefore treatment differences were analysed non-parametrically using the Kruskall-Wallis test. For the first two milkings post-deprivation, it was not possible to discern whether four hours of lying deprivation had an effect on milk yield by examining individual treatment differences. Thus, data for treatments C and 2 were combined to determine whether milk yields for 0 – 2 h of lying deprivation were significantly different from 4 h, during these times. When the milk yield data were combined in this way, only the afternoon milk recordings were normally distributed for both the NMR and post-deprivation recordings.

5.3.2a Continuous recordings

As the behaviours observed were mutually exclusive, it was possible to combine behaviours to form new behavioural categories. For example, sniffing the ground and sniffing the housing were combined to form the new category 'sniffing'. The individually recorded behaviours combined to form new behavioural categories are listed in Table 5.5a. The experimenter labelled some of the new behavioural categories with a word that was considered to most appropriately describe the probable emotional state an animal was experiencing when performing the individual behaviours listed within a combination. These categories, *i.e.* restlessness, stress, and frustration, can thus be considered as subjective combinations of individual behaviours. The grouping of certain behaviours were based on the expectation that they would vary together, in the same direction, with time. The term stress is defined here as "an environmental effect on an individual which overtaxes its control systems and reduces its fitness or appears likely to do so" (Broom & Johnson, 1993). Broom & Johnson (1993) also stated that frustration results due to an inability to perform a behaviour that the animal is motivated to do. Thus, the stressor, in this study, was the deprivation of lying, which may result in frustration if the cow was motivated to lie during the deprivation period. As lying deprivation can be considered as stressful and frustrating, those behaviours increasing in frequency with time in, or as a result of this activity, can be considered as behavioural responses to this situation and therefore labelled accordingly. In addition, various researchers have demonstrated that there are certain behaviours, which under certain conditions, can be indicative of stress and frustration. For example, Sandem *et al.* (2002) stressed dairy cows by subjecting them to a frustrating situation (food deprivation) and found that they showed aggression (*i.e.* butting), vocalisation, and head swinging activity, which were not observed in the

control cows. These behaviours can thus be described as outward expressions of the animals' negative mental state. Vocal behaviour has also been demonstrated as an indicator of frustration and stress in other studies (Dunn, 1990; Watts & Stookey, 2000; Zimmerman *et al.*, 2000), and head swinging activity has been observed in other animals experiencing frustrating situations (Cook, 1992).

Table 5.5a The new behavioural categories, and the individually recorded behaviours which were combined to form them

New behavioural category	Individual behaviours combined to form new category
Shifting	Leg raising; repositioning; weight shifting.
Restlessness	Leg raising; repositioning; weight shifting; head swinging
Sniffing	Sniff ground; sniff housing
Body care	Grooming self; rubbing head against housing
Housing interaction	Rubbing head against housing; sniff housing; lick housing
Licking	Grooming self; lick housing
Stress	Leg raising; repositioning; weight shifting; head swinging; butting; vocalising; grooming self
Frustration	Head swinging; butting; vocalising

Aggression can be defined as "a physical act or threat of action by an individual which causes pain or injury or reduces freedom in another individual" (Broom & Johnson, 1993). It can be considered as the behavioural display of an animal's willingness to overcome a negative situation (Sandem *et al.*, 2002) and performed as a result of a frustrative state (Dollard *et al.*, 1939; Miller *et al.*, 1941; Scott, 1948; Duncan & Wood-Gush, 1971). Thus, the individually combined behaviours can be considered as indicative of the mental state the animal was likely to be experiencing during lying deprivation. The situation the animal is in is an important consideration

in behavioural interpretation, as vocalisations per se, for example, are not necessarily indicative of negative psychological welfare. Vocalisation can also be used to communicate levels of excitement (Phillips, 1993).

The following behaviours were not observed: Feed tossing, leaning against housing, licking ground, lying, nosing ground, pushing, rubbing body against housing, rubbing body against other, grooming other, and threat. Licking housing and vocalising had too few observations for any useful statistical analysis to be carried out. All the data recorded for each behaviour were discrete and had low frequencies (less than 29 possible values) thus were not suitable to analyse parametrically.

Each behavioural variable was first tested for differences between hours, for each treatment. For treatment C, all behaviours, except walking, body care, licking and stress, had greater than 50% zero values for one or more hours. All behaviours, except body care, licking, restlessness, stress and housing interaction, had greater than 50% zero values for one or more hours, for treatment 2. For treatment 4, all behaviours, except weight shifting, repositioning, grooming self, drinking, body care, licking, frustration, sniffing, housing interaction, time spent drinking, number of licks whilst grooming self, shifting, restlessness and stress, had greater than 50% zero values for one or more hours. Where the data for those variables had greater than 50% zero values for one or more hours, the 1-Sample Sign test was used to determine the true median value for each hour (Snedcor & Cochrane, 1978). This provides a meaningful comparison between hours for each treatment. For the remaining behaviours, except shifting, restlessness and stress for treatment 4, differences between hours were analysed using the Kruskall-Wallis test. For treatment 4, data for stress (for each

hour), shifting and restlessness (for hours two, three and four) followed normal distributions. For the first hour of both shifting and restlessness it was necessary to use logarithms of the original data to achieve normal distributions. The variances were homogenous between all hours for each behaviour. These behaviours were therefore analysed by ANOVA. For those behaviours where two of the hours had less than 50% zero values, differences between these hours were also analysed using the Mann-Whitney test. These behaviours were the number of licks whilst grooming self (treatments C and 2) and the time spent rubbing head against furniture (treatments C and 4).

Each behavioural variable was then tested for differences between treatments for each hour, and for the average frequency/duration of the activity for the entire four hour deprivation period. For each hour, all the individual behaviours, except drinking, time spent drinking (first hour for both), time spent rubbing head, number of licks whilst grooming self, grooming self, rubbing head against housing (third hour for all) and walking (hours one and two), had greater than 50% zero values for at least one treatment. For each hour, all the combined behaviours, except shifting, frustration (hours one, two and three for both), housing interaction (hours two and four), restlessness (hour three), and sniffing (hour four), had less than 50% zero values for all treatments recorded performing the behaviours. All behaviours for the average frequency/duration over the entire deprivation period, except butting, sleeping and time spent sleeping, had less than 50% zero values for each treatment recorded performing the behaviour. Those behaviours with greater than 50% zero values, for at least one of the treatments, were analysed for treatment differences using the 1-Sample Sign test. For all other behaviours, treatment differences were analysed using

the Mann-Whitney test where only two treatments were recorded performing the behaviour, *e.g.* walking, or the Kruskall-Wallis test for all three treatments. For those behaviours where two of the treatments had less than 50% zero values, treatment differences were also analysed using the Mann-Whitney test. These behaviours were weight shifting (hours three and four), shifting (hour two), restlessness (hour three), and housing interaction (hour two).

Spearman's rank order correlation coefficients were determined between each behaviour (using the average frequency/duration of a behaviour over the entire deprivation period) and milk yield (NMR data), the number of days in milk, the number of days to parturition, body condition score and lactation number, for each treatment. The Spearman's rank order correlation was selected over the Pearson's correlation as the data was not continuous or normally distributed (Dytham, 1999). A linear regression analysis was performed on statistically significant ($P \leqslant 0.05$) correlations. There was no evidence for non-linearity for significant ($P \leqslant 0.05$) regressions. Only those behaviours significantly correlated with at least one treatment are shown and discussed in the results section.

5.3.3a Instantaneous recordings

As the behaviours observed were mutually exclusive, it was possible to combine behaviours to form new behavioural categories. The three new behavioural categories examined, and the individual behaviours combined to form them, are shown in the results section (Table 5.29a).

Each behaviour was tested for differences between hours, for each treatment, and between treatments, for each hour, using either ANOVA, Kruskall-Wallis, Mann-Whitney or the 1-Sample Sign test, depending on the nature of the data. ANOVA was used where the data for two or more comparisons were normally distributed. The Kruskall-Wallis test was selected where the data for three (between hours/treatments) or more (between hours only) comparisons were not normally distributed. Where the data for two comparisons were not normally distributed and resistant to mathematical transformation to normality, the Mann-Whitney test was selected. The 1-Sample Sign test was employed when the data for one or more comparisons had greater than 50% zero values. More than one test may have been carried out on the data for comparisons between treatments/hours. Where appropriate to test, all data for statistical comparisons between hours and treatments had equal variance.

For treatment C, all behaviours, for each hour, followed a normal distribution except feeding (hours two, three and four), standing (all hours), lying (hour one), standing ruminating (all hours), lying ruminating (hours one and three), and all standing behaviours (hours two and four). For standing ruminating, data for each hour had greater than 50% zero values. For treatment 2, all behaviours for each hour were normally distributed except lying, standing ruminating (both hours one and two), and lying ruminating (hour one). For standing ruminating, data for both hours had greater than 50% zero values. For treatment 4, all behaviours for each hour were normally distributed except feeding (hour 4), standing (hour 4), standing ruminating (hours one and two), and all standing behaviours (hour four). For all treatments, all non-normal data were resistant to mathematical transformation to normalisation except for the third hour for all standing behaviours (treatment C), the second hour for lying

(treatment 2), and standing (treatment 4), which were transformed using logarithms of the original data. For each treatment, the data for the total time spent in a behaviour, for all four hours combined, followed normal distributions.

Pearson's correlation coefficients were determined between each behaviour (using the data for the total time spent in a behaviour for all four hours) and production variables (see continuous recordings correlations for variables), for each treatment. A linear regression analysis was performed on statistically significant ($P \leqslant 0.05$) correlations. There was no evidence for non-linearity for significant ($P \leqslant 0.05$) regressions.

5.3.4a Lying position and changes in posture post-deprivation

Due to a slight change in milking times, the cows were only observed for 50 min instead of the scheduled hour.

A Chi-square test of association was employed to determine the effect of lying side on deprivation length. This test makes no assumptions about the form of the data and is suited for analysis of frequencies (Dytham, 1999). None of the expected values were less than five.

5.3.5a Post-deprivation video observations

The mean time spent in each behaviour per hour for each treatment was calculated and displayed graphically to determine whether there were any obvious differences between treatments in the behaviours performed over time. To ensure equal observation times for all cows in each block per hour, data were omitted so all blocks had the same start and finish times for each milking. This resulted in data being

omitted for hours 15.25 and 16.25 on day one, and 06.25, 07.25, 15.25 and 16.25 on day two, *i.e.* hours 2, 3, 17, 18, 26 and 27, respectively. Also, the first 15 min of data were discounted for hours 14.25 (hour one) and 08.25 (hour 19), and the first 5 min for hours 05.25 (hour 16) and 08.25 (hour 25). The graphs revealed that eight hour periods would best suit the data for further exploration.

The mean time spent in each behaviour per eight hours for the 41 h period, the entire 41 h period (hours 1 to 41), and the entire 41 h period including the four hour deprivation period (hours - 4 to 41) was calculated for each treatment. Milking times were made equal for all blocks by discounting any data recorded immediately post-milking, thus ensuring all blocks were observed for the same amount of time. Data collected post-milking was discounted rather than that collected prior to milking, as the cows were considered more likely to be unsettled during this period. Data for each block of cows were discounted to equal the longest milking time for a block of cows within that milking session. The longest first, second and third milking session was 65, 75 and 60 min, respectively. These milkings took place between hours 15.10 to 17.00 (hours 1–3), 06.20 to 08.35 (hours 16–19) and 15.25 to 17.15 (hours 25-27), respectively. The fourth milking took place when the cows were returned to the herd, therefore did not affect the total observation time between blocks of cows. All data for between treatment comparisons, for each behaviour, in each time period, had equal variance. For treatment C, all behaviours for each time period followed a normal distribution, except feeding (hours 9-16), standing (hours 9-16, 17-24 and 33-40) and lying (hours 9-16). For treatment 2, all behaviours for each time period were normally distributed, except feeding (hours 33-40) and standing (hours 33-40, 1-41 and - 4-41). For treatment 4, all behaviours for each time period were normally distributed, except

feeding (hours 9-16), standing (hours 9-16, 17-24, and 33-40) and lying (hours 33-40). All non-normal data for all treatments, except lying (treatment C, hours 9-16) and standing (all treatments, hours 33-40), were mathematically transformed using logarithms of the original data to obtain approximate normal distributions. The data for lying behaviour for treatment C, during hours 9-16, were cube transformed to obtain a normal distribution. The data for standing, for all treatments, during hours 33-40, were resistant to mathematical transformation to normality. Each behavioural variable, for each time period, was tested for treatment differences by ANOVA providing the values were normally distributed. For standing behaviour, during hours 33-40, treatment differences were determined using the Kruskall-Wallis test for non-parametric analysis.

Pearson's correlation coefficients were determined between each behaviour (using the mean time spent in a behaviour for the first eight hours) and production variables (see continuous recordings correlations for variables) for each treatment. A linear regression analysis was performed on statistically significant ($P \leqslant 0.05$) correlations. There was no evidence for non-linearity for significant ($P \leqslant 0.05$) regressions.

The latency to, and duration of, the first lying bout immediately following the first milking post-deprivation, was calculated for each treatment. Data for the duration of lying were homogenous and normally distributed for each treatment. Treatment differences were therefore determined by ANOVA. For latency to lying, no treatment had data following a normal distribution, and data for treatment 2 were resistant to mathematical transformation procedures. Thus, treatment differences were determined using the Kruskall-Wallis test.

5.4a Results

5.4.1a Health status of animals during the study

All cows maintained good health for the duration of the study, in particular they had no obvious signs of mastitis or clinical lameness.

5.4.2a Milk yield recordings

5.4.2.1a Pre-experimental milk yields (control recordings)

There was a tendency for treatment 4 cows to have a slightly lower morning and total (morning + afternoon) pre-experimental mean milk yield in comparison to the cows in treatments C and 2, which were similar (P=0.07; P=0.06, respectively) (Table 5.6a). There was no significant difference in the pre-experimental mean milk yields between treatments during the afternoon milking session.

Table 5.6a The morning (am), afternoon (pm) and total (am+pm) pre-experimental (NMR) mean milk yields (kg) for the experimental cows used in the control (C), 2 h (2) and 4 h (4) treatments

Milking	Treatment C	2	4	SED ±	P-value
am	15.5	15.9	13.7	0.97	0.07
pm	9.4	9.1	8.8	0.51	0.58
Total (am + pm)*	25.6 (655.7)	25.7 (662.2)	23.1 (532.7)	(58.03)	0.06

*Used back transformed data to calculate values. Transformed values in parenthesis

5.4.2.2a Post-deprivation milk yields

Cows in treatment 4 had a lower mean milk yield in comparison to those in treatment 2, during the first milking post-deprivation (P=0.02) (Table 5.7a(i)). The Tukey's

post-hoc test revealed that there was no significant difference in mean milk yield between the treatment C cows and those in treatments 2 or 4, at this time. There was no significant difference in mean milk yields between treatments during the following milking and when the data for these two milkings were combined (Table 5.7a(i)).

When the cows in treatments C and 2 were combined, they had a significantly greater mean milk yield in comparison to the treatment 4 cows, for the first milking post-deprivation (Table 5.7a(ii)). There was no significant difference in mean milk yield between these two treatments during the following milking or for the total mean milk yield for these two recordings (Table 5.7a(iii)).

Table 5.7a Mean post-deprivation milk yield values (kg) for all the experimental cows used in the control (C), 2 h (2) and 4 h (4) treatments:

(i) The first (pm) and second (am) milkings, and their total (pm+am)

Milking	Treatment			SED ±	P-value
	C	2	4		
First (pm)	8.8	9.5	8.3	0.38	**0.02**
Second* (am)	15.9 (252.2)	15.5 (240.5)	15.8 (250.5)	(14.88)	0.72
Total* (pm + am)	24.7 (612.0)	25.3 (642.4)	24.5 (602.0)	(32.30)	0.47

*Used back transformed data to calculate values. Transformed values in parentheses

(ii) The first (pm) and second (am) milkings, and their total (pm+am) for the control and 2 h treatments when combined (C+2) and the 4 h treatment (4)

Milking	Treatment		SED ±	P-value
	C+2	4		
First (pm)	9.2	8.3	0.36	**0.02**
Second* (am)	15.6 (244.8)	15.9 (253.5)	(13.38)	0.54
Total* (pm + am)	25.0 (624.9)	24.6 (606.6)	(31.52)	0.58

*Used back transformed data to calculate values. Transformed values in parentheses

(iii) The third (pm) and final (am) milkings, and their total (pm+am)

Milking	Treatment			SED ±	P-value
	C	2	4		
Third (pm)	8.9	8.9	8.0	1.6	0.32
Fourth* (am)	14.7 (1.168)	14.7 (1.167)	13.4 (1.126)	(0.0255)	0.43
Total (pm + am)	24.3	24.4	21.9	2.3	0.24

*Used back transformed data to calculate values. Transformed values in parenthesis

(iv) The combined afternoon (pm) (1st and 3rd milking) and morning (am) (2nd and 4th milking) recordings, and the mean for all four occasions (pm+am)

Milking	Treatment			SED ±	P-value
	C	2	4		
pm	9.0	9.2	8.0	1.57	0.14
am	16.0 [m]	16.8 [m]	13.2 [m]	-	0.15
pm + am	24.5	24.8	22.1	2.31	0.16

[m]Median values

There was no significant difference between any of the treatments for the mean milk yields taken during the third or fourth milking, or for the mean total of these two recordings (Table 5.7a(iii)).

The average milk yield over the two days for cows in all three treatments was similar for the combined afternoon measurements (P=0.14), combined morning recordings (P=0.15), and the total of the morning and afternoon values (P=0.16) (Table 5.7a(iv)).

5.4.3a Behavioural observations taken during the deprivation period

No cow from either of the deprivation groups attempted to lie down during actual deprivation.

5.4.3.1a Continuous recordings

Each sub-table (1 to 24) in Table 5.8a shows the significance of the differences between values for each hour (by row) and for each treatment (by column), for a specific behaviour.

Between hours, leg raising was not observed to any major extent for cows in treatment C. There was a significant incidence of leg raising for the first hour of deprivation for cows in both treatments 2 and 4 (Table 5.8a1). There was an increase in the second hour of deprivation for treatment 4 cows, to a level which was maintained for the third and fourth hour.

Table 5.8a The average frequency (no. h^{-1}), or duration (sec h^{-1}), that each behaviour was performed per hour of the deprivation period, and the average per hour for all four hours, for all of the experimental cows in the control (C), 2 h (2) and 4 h (4) treatments

1. Leg raising (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{c}0^{a}$	$_{b}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	$_{b}0^{a}$	-	0.3
2	$_{b}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	$_{a}1.5^{a}$	$_{a}2.5^{a}$	-	1.1
4	$_{a}1^{b}$	$_{a}3.0^{a}$	$_{a}2.5^{a}$	$_{a}3.0^{a}$	-	3.0
P-value between treatments	-	-	-	-		**<0.01**

2. Repositioning (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	-	-	-	-	-	-
2	-	-	$_{a}2.5^{a}$	$_{a}4.0^{a}$	-	1.9
4	2.0	4.0	$_{a}4.5$	$_{a}5.0$	**0.02**	3.1
P-value between treatments	-	-	-	-		**<0.01**

3. Weight shifting (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}0^{b}$	$_{b}0^{b}$	$_{c}0^{b}$	$_{c}0\text{-}1^{a}$	-	0.1
2	$_{b}0^{c}$	$_{b}0\text{-}1^{b}$	$_{b}3.0^{a}$	$_{b}4.0^{a}$	-	2.3
4	$_{a}2.0$	$_{a}4.5$	$_{a}6.0$	$_{a}7.5$	**<0.01**	6.1
P-value between treatments	-	-	(2 & 4)[1] **0.04**	(2 & 4)[1] **0.03**		**<0.01**

[1]Numbers in parenthesis represent the treatments to which the P-value refers

4. Shifting* (no. h^{-1})	Hour 1	2	3	4	SED ±	P-value between hours	Median value for all hours
C	$_{c}0^{c}$	$_{b}1.0^{a}$	$_{c}0\text{-}1^{b}$	0.5^{a}	-	-	0.8
2	$_{b}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	$_{b}8.5^{a}$	11.5^{a}	-	-	5.5
4 [(m)]	5.3^{b} $_{a}5.5$	11.7^{a} $_{a}13.5$	13.1^{a} $_{a}14.0$	18.1^{a} 20.0	3.62	**<0.01**	12.1
P-value between treatments	-	-	(2 & 4)[1] **0.02**	**<0.01**			**<0.01**

*Leg raising + repositioning + weight shifting; [m]mean values on top row; [1]Numbers in parenthesis represent the treatments to which the P-value refers

5. Head swinging (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}0\text{-}1^{a}$	$_{b}0\text{-}1^{a}$	$_{b}0^{b}$	$_{b}0^{b}$	-	0.3
2	$_{c}0^{b}$	$_{c}0^{b}$	$_{a}0\text{-}1^{a}$	$_{a}0\text{-}1^{a}$	-	0.3
4	$_{a}0.5^{a}$	$_{a}1.0^{a}$	$_{a}0\text{-}1^{b}$	$_{a}0\text{-}1^{b}$	-	0.5
P-value between treatments	-	-	-	-		0.09

6. Restlessness* (no. h^{-1})	Hour 1	2	3	4	SED ±	P-value between hours	Median value for all hours
C	0.5^{b}	1.5^{a}	$_{c}0\text{-}1^{b}$	1.0^{a}	-	-	1.0
2	0.5	1.5	$_{b}9.0$	12.0	-	**<0.01**	6.1
4 [(m)]	6.4^{b} 7.5	13.0^{a} 15.5	14.5^{a} $_{a}14.5$	19.6^{a} 23.0	3.50	**<0.01**	16.0
P-value between treatments	**<0.01**	**<0.01**	(2 & 4)[1] **0.01**	**<0.01**			**<0.01**

*Leg raising + repositioning + weight shifting + head swinging; [m]mean values on top row; [1]Numbers in parenthesis represent the treatments to which the P-value refers

7. Sniffing ground (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{a}1.0^{a}$	$_{b}0\text{-}1^{b}$	$_{a}1.0^{a}$	$_{a}1.0^{a}$	-	0.6
2	$_{a}1.0^{a}$	$_{a}0.5^{a}$	$_{b}0^{c}$	$_{b}0\text{-}1^{b}$	-	0.5
4	$_{b}0\text{-}1^{a}$	$_{a}1.0^{a}$	$_{c}0\text{-}1^{a}$	$_{b}0\text{-}1^{a}$	-	0.3
P-value between treatments	-	-	-	-		0.09

8. Sniffing housing (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}0\text{-}1^{a}$	$_{a}0\text{-}1^{a}$	$_{b}0^{b}$	$_{c}0^{b}$	-	0.3
2	$_{a}1.0^{a}$	$_{a}0\text{-}1^{b}$	$_{a}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	-	0.5
4	$_{b}0\text{-}1^{b}$	$_{a}0\text{-}1^{b}$	$_{a}0\text{-}1^{b}$	$_{a}1.0^{a}$	-	0.5
P-value between treatments	-	-	-	-		0.36

9. Sniffing housing (sec h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{a}1.0^{a}$	$_{a}0\text{-}1^{b}$	$_{b}0^{c}$	$_{c}0^{c}$	-	1.1
2	$_{a}1.0^{a}$	$_{a}0\text{-}1^{b}$	$_{a}1.0^{a}$	$_{b}0\text{-}1^{b}$	-	2.1
4	$_{a}1.0^{a}$	$_{a}1.0^{a}$	$_{a}1.0^{a}$	$_{a}3.0^{a}$	-	2.3
P-value between treatments	-	-	-	-		0.63

10. Sniffing* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0^{a}	1.0^{a}	1.0^{a}	$_{a}1.0^{a}$	-	1.3
2	1.0^{a}	1.0^{a}	1.0^{a}	$_{a}1.0^{a}$	-	1.1
4	1.0	1.0	1.0	$_{a}1.5$	0.50	1.1
P-value between treatments	0.24	0.93	0.91	-		0.78

*Sniffing ground + sniffing housing

11. Grooming self (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	0.5 [a]	1.0 [a]	1.0 [a]	0-1 [b]	-	1.0
2	2.0 [a]	0-1 [b]	1.0 [a]	0-1 [b]	-	0.9
4	0.5	1.0	1.0	0.5	0.18	1.1
P-value between treatments	0.30	-	1.0	-		0.73

12. Grooming self #	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0 [a]	3.0 [a]	4.5 [a]	1.0 [a]	(2&3)[1] 0.38	4.1
2	2.0 [a]	1.0 [a]	4.0 [a]	1.0 [a]	(1&3)[1] 0.84	4.5
4	1.5	4.0	3.0	2.0	0.96	6.5
P-value between treatments	-	-	0.95	-		0.41

Total number of licks; [1]Numbers in parenthesis represent the hours to which the P-value refers

13. Rubbing head against housing (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0 [a]	0-1 [b]	0.5 [b]	0-1 [b]	-	0.5
2	0-1 [b]	0-1 [b]	0.5 [a]	0-1 [b]	-	0.8
4	1.0 [a]	1.0 [a]	1.0 [a]	0-1 [b]	-	0.8
P-value between treatments	-	-	0.56	-		0.60

14. Rubbing head against Housing (sec h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	3.5 [a]	0-1 [b]	2.0 [a]	0-1 [b]	(1&3)[1] 0.90	4.1
2	1.0 [a]	0-1 [b]	1.0 [a]	1.0 [a]	-	2.5
4	1.0 [a]	4.0 [a]	5.5 [a]	0-1 [b]	(2&3)[1] 0.81	5.4
P-value between treatments	-	-	0.77	-		0.37

[1]Numbers in parenthesis represent the hours to which the P-value refers

15. Body care* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0	1.0	2.0	1.0	0.21	1.5
2	1.0	1.0	2.0	1.0	0.36	1.4
4	2.0	2.5	2.0	1.0	0.16	2.0
P-value between treatments	0.79	**0.03**	0.78	0.54		0.49

*Grooming self + rubbing head against furniture

16. Housing interaction* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0 [a]	[c] 0-1 [b]	3.0 [a]	[a] 0-1 [b]	-	1.0
2	1.0	[b] 1.0	1.0	[a] 1.0	0.39	1.3
4	1.0	[a] 2.0	1.0	[a] 1.0	0.35	1.8
P-value between treatments	0.99	(2 & 4)[1] **0.01**	0.18	-		0.18

*Rubbing head against housing + sniffing housing + licking housing; [1]Numbers in parenthesis represent the treatments to which the P-value refers

17. Licking* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0	[b] 1.0	2.0	1.0	0.62	1.9
2	2.0	[b] 1.0	2.0	1.0	0.36	1.6
4	2.5	[a] 3.0	3.0	2.0	0.23	3.3
P-value between treatments	0.51	**0.01**	0.08	0.31		0.06

*Grooming self + licking housing

18. Stress* (no. h^{-1})	Hour 1	2	3	4	SED ±	P-value between hours	Median value for all hours
C	1.5	2.0	1.5	2.0	-	0.50	2.2
2	3.0	2.0	10.5	14.0	-	**<0.01**	8.0
4 [(m)]	12.0 [b] 11.0	17.6 [a,b] 18.5	18.8 [a,b] 18.5	24.4 [a] 24.5	3.18	**<0.01**	18.6
P-value between treatments	**<0.01**	**<0.01**	**<0.01**	**<0.01**			**<0.01**

*Leg raising + repositioning + weight shifting + head swinging + butting + vocalising + grooming self; [m]mean values on top row

19. Butting (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}0^{a}$	$_{a}0^{a}$	$_{b}0^{a}$	$_{b}0^{a}$	-	$_{b}0.0$
2	$_{b}0^{b}$	$_{a}0^{b}$	$_{a}0\text{-}1^{a}$	$_{a}0\text{-}1^{a}$	-	$_{a}0.1$
4	$_{a}0\text{-}1^{a}$	$_{a}0^{b}$	$_{a}0\text{-}1^{a}$	$_{a}0\text{-}1^{a}$	-	$_{a}0.3$
P-value between treatments	-	-	-	-		-

20. Frustration* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}0\text{-}1^{b}$	$_{a}1.0^{a}$	$_{b}0^{c}$	$_{b}0^{c}$	-	0.4
2	$_{b}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	$_{a}1.0^{a}$	$_{a}1.0^{a}$	-	0.9
4	$_{a}1.0$	$_{a}1.0$	$_{a}1.0$	$_{a}1.0$	0.95	1.3
P-value between treatments	-	-	-	-		**0.01**

*Head swinging + butting + vocalising

21. Drinking (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	0.5^{b}	$_{a}1.0^{a}$	$_{b}0\text{-}1^{c}$	$_{a}1.0^{a,c}$	-	0.8
2	1.0^{a}	$_{b}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	-	0.6
4	1.0	$_{a}1.0$	$_{a}2.0$	$_{a}0.5$	0.13	1.5
P-value between treatments	0.52	-	-	-		**<0.01**

22. Drinking (sec h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	*3.5*^{a}	*$_{b}1.0^{b}$*	*$_{b}1.0^{b}$*	*$_{a}16.0^{a}$*	-	13.8
2	*8.5*^{a}	*$_{b}1.0^{a}$*	*$_{b}1.0^{a}$*	*$_{a}1.0^{a}$*	-	8.6
4	10.0	$_{a}14.0$	$_{a}33.5$	$_{a}2.5$	0.13	20.0
P-value between treatments	0.61	-	-	-		***0.05***

23. Sleeping[¶]	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_a$0[b]	$_a$0-1[a]	0-1[a]	0[b]	-	$_a$0-1
2	$_a$0[b]	$_a$0-1[a]	-	-	-	$_a$0-1
4	-	-	-	-	-	-
P-value between treatments	-	-	-	-		-

[¶]Table represents the results for both frequency and duration (min h^{-1}) of sleeping

24. Walking (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0	1.0	1.0	1.5	0.72	1.8
2	1.0[a]	1.0[a]	-	-	-	0.5
4	-	-	-	-	-	-
P-value between treatments	0.75	0.78	-	-		**<0.01**

Key:

- Row values with different superscripts are significantly different ($P \leqslant 0.05$).
- Treatment values with different subscripts are significantly different ($P \leqslant 0.05$).
- Where letters to indicate differences between values are absent, but a significant probability value has been shown, a non-parametric test was performed and therefore no post-hoc test of paired means could be carried out.
- All values are medians unless otherwise stated. Mean values were only used to determine differences between hours.
- The symbol '-' in the treatment/hour box indicates that the cows for that treatment were unable to perform the behaviour in that hour.
- '0-1' denotes the median is zero, but the 1-Sample Sign test indicates that the true median lies between zero and one.
- Bold P-values are used where $P \leqslant 0.05$

There was a small but significant increase in leg raising between treatments in the first hour, with the cows in treatment 4 having the greatest frequency and those in treatment C the lowest (Table 5.8a1). In the second hour, treatment 4 cows had a significantly higher rate of leg raising in comparison to the cows in the other two treatments, which were not significantly different. Cows in treatments 2 and 4 raised their legs significantly more often than those in treatment C in the last two hours. There was no significant difference between treatment 2 and 4 cows during these times. Over the entire deprivation period, leg raising increased significantly between treatments with increasing deprivation length.

Repositioning was not observed, and weight shifting was not observed to any major extent, for the cows in treatment C (Table 5.8a2 & 3). Both behaviours were performed more frequently between hours during deprivation for cows in treatments 2 and 4, and for cows in treatment 4 these behaviours increased with the duration of deprivation.

There was no significant difference in repositioning between treatments in the last two hours (Table 5.8a2). Weight shifting significantly increased between the cows in treatments 2 and 4 with increasing deprivation length in all hours (Table 5.8a3). Both behaviours significantly increased between treatments with increasing deprivation time over the entire four hour period.

Between hours, shifting behaviours were not observed to any major extent in treatment C. There was an increase in shifting behaviours for the first hour of deprivation for cows in both treatments 2 and 4 (Table 5.8a4). There was a further

increase in the second hour of deprivation for cows in these two treatments and a continued increase in the third and fourth hour for treatment 4 cows.

The number of times observed shifting increased significantly between treatments with increasing deprivation time in all hours except the second (Table 5.8a4). In the second hour, treatment 4 performed this behaviour significantly more than the other two treatments, which were not significantly different from each other. Over the whole of the deprivation period, the frequency of shifting was significantly different between treatments and increased with increasing deprivation length ($P < 0.01$).

Head swinging was rarely observed but there was a tendency for it to increase between hours mainly in the first two hours of deprivation for cows in treatments 2 and 4 (Table 5.8a5). There were no major differences between treatments in head swinging for any of the hours. However, there was a tendency for cows in treatment 4 to perform this activity more frequently than the other two treatments over the entire deprivation period ($P=0.09$).

Restlessness type behaviours were only observed to any major extent in the second hour for treatment C cows (Table 5.8a6). In both treatments 2 and 4, there was an increase in restlessness type behaviours between hours during the deprivation period ($P < 0.01$; $P < 0.01$, respectively). For the treatment 2 cows, the greatest increase took place in the first hour of deprivation. In treatment 4 cows, only the first hour was significantly different from the remaining three, which were not significantly different from each other.

Cows in treatments C and 2 had a median frequency of 0.5 and 1.5 for restlessness type behaviours for the first and second hour of the deprivation period, respectively (Table 5.8a6). Treatment 4 cows carried out these behaviours significantly more frequently than the cows in the other treatments during these hours. In the third and fourth hours, this activity increased in frequency between treatments with increasing deprivation time ($P=0.01$; $P<0.01$, respectively). Over the entire four hour period, treatment 4 cows were most restless and treatment C cows the least ($P<0.01$).

The incidence of ground sniffing was low for cows in all treatments and tended to decline between hours during lying deprivation (Table 5.8a7). Generally, ground sniffing frequency was higher in treatment C cows than cows in treatment 2 (during deprivation) and 4. Over the course of the deprivation period, there was a tendency for ground sniffing to decrease, between treatments, with increasing deprivation time ($P=0.09$).

The incidence of, and time spent, sniffing the housing and grooming self was low for cows in all treatments and not clearly affected by time or treatment (Tables 5.8a8, 9 and 11, respectively)

There was no significant difference between hours for sniffing behaviours and the total number of licks whilst self grooming for any of the three treatments (Table 5.8a10 and 12, respectively). There was no significant difference between treatments for any of the hours, or over the entire deprivation period, for these behaviours.

The frequency of, and time spent, rubbing the head against the housing was not clearly affected by time or treatment (Tables 5.8a13 and 14, respectively).

There was no difference in the frequency of body care type behaviours performed over time for cows in treatments C, 2 or 4 (P=0.21; P=0.36; P=0.16, respectively) (Table 5.8a15). There was also no significant difference between treatments for hours one, three and four, or over the entire deprivation period. In the second hour, treatment 4 cows performed these behaviours more frequently than cows in treatments C and 2, which were both observed in these activities a median of 1.0 (P=0.03).

The incidence of housing interaction was low for cows in all treatments (Table 5.8a16) and there were no major differences between hours in the performance of this behaviour for cows in all treatments.

There were no significant differences between treatments in housing interaction frequency for the first, third or fourth hour, or over the entire deprivation period (Table 5.8a16). Treatment had a significant effect on this behaviour in the second hour, with treatment C cows observed interacting the least and treatment 4 cows the most.

There was no difference in the frequency of licking behaviours performed over time for cows in treatments C, 2 or 4 (P=0.62; P=0.36; P=0.23, respectively) (Table 5.8a17).

In the second hour, licking behaviours were performed significantly more frequently by cows in treatment 4, compared with the other two treatments, which were not significantly different from each other (Table 5.8a17). There were no significant differences between treatments for any other hour. For the whole of the deprivation period, there was a tendency for cows in treatment 4 to perform these behaviours more frequently than those in the other two treatments (P=0.06).

There was no difference between hours in the performance of stress type behaviours for treatment C cows (P=0.50) (Table 5.8a18). Treatment 2 cows increased the frequency of this behaviour during actual deprivation (hours 3 & 4) ($P < 0.01$). Cows in treatment 4 increased the frequency of these stress type behaviours over time. However, only the first and fourth hours were significantly different from each other ($P < 0.01$).

The frequency of stress type behaviours were significantly different between treatments and increased with increasing deprivation length for all hours, and over the entire four hour period (Table 5.8a18).

Cows in treatment C were not observed butting (Table 5.8a19). Butting was rarely observed for the cows in the other two treatments. However, the median frequency of butting increased between hours during actual deprivation for cows in treatment 2. Treatment 4 cows were observed butting in all hours (median of 0-1) except the second.

Only treatment 4 cows were observed butting in the first hour. Cows in all three treatments were not observed butting in the second hour (Table 5.8a19). There was no difference in the frequency of butting between cows in treatments 2 and 4 in the last two hours of the deprivation period, or over the entire period.

Treatment C cows did not perform frustration type behaviours to any major extent (Table 5.8a20). The frequency of frustration type behaviours increased between hours during actual deprivation for treatment 2 cows. Cows in treatment 4 had a median value of 1.0 for each hour (P=0.95).

Cows in treatment 4 were observed significantly more often in frustration type behaviours during the first hour, in comparison to the cows in treatments C and 2, which were not significantly different (Table 5.8a20). In the second hour, only treatment 2 cows were significantly lower in comparison to cows in the other two treatments, which were not significantly different. There was no significant difference between cows in treatments 2 and 4 in the third or fourth hour, but cows in both treatments performed this behaviour significantly more frequently than cows in treatment C in both hours. Over the entire four hour period, the frequency of frustration type behaviours were significantly different between treatments, and increased with increasing deprivation time (P=0.01).

The median frequency of drinking between hours was low for cows in all treatments (Table 5.8a21). The number of times the cows in each treatment were observed drinking was not different in the first hour (P=0.52) and there was no clear effect of treatment on drinking frequency in any of the remaining hours. The frequency of

drinking was greater in treatment 4 cows, than cows in treatments C and 2, over the entire deprivation period ($P < 0.01$).

For treatment C cows, the time spent drinking was significantly greater in the first and fourth hours, which were not significantly different, compared with the second and third hours, which were also not significantly different (Table 5.8a22). There was no significant difference between hours in this behaviour for cows in treatments 2 or 4.

No treatment effect was observed for time spent drinking in the first or final hour (Table 5.8a22). In the second and third hour, treatment 4 cows drank for significantly longer than cows in treatments C and 2, which were not significantly different from each other. Cows in treatment 4 spent the most amount of time drinking followed by cows in treatment C over the course of the deprivation period ($P=0.05$).

Cows in treatments 2 and 4 were not recorded sleeping during actual deprivation (Table 5.8a23). The frequency and time spent sleeping was not observed to any major extent in the treatment C or 2 cows.

Cows in treatments 2 and 4 were restricted from walking during actual deprivation (Table 5.8a24). There was no significant difference between hours in walking activity for cows in either treatment C or 2.

No significant difference was observed between treatments C and 2 in the first or second hour for walking frequency (Table 5.8a24). Cows in treatment 2 were observed walking less over the entire period ($P < 0.01$).

5.4.3.2a Behavioural versus production variable correlations for continuous recordings

Cows in treatment 4 increased leg raising behaviour with increasing number of days in milk (r^2=27.7, P=0.02) (Table 5.9a). There was a negative correlation between this behaviour and body condition score for treatment C cows (r^2=26.8, P=0.02) (Table 5.11a). The frequency of leg raising was negatively correlated with milk yield for treatment 4 cows (P=0.04), and there was a tendency for these variables to be positively correlated for the cows in treatment C (P=0.06) (Table 5.12a).

Cows in treatment 2 had a negative correlation between repositioning and the number of days to parturition (P=0.01) (Table 5.13a).

Weight shifting was positively correlated with the number of days in milk for treatment 4 cows (r^2=28.4, P=0.02) (Table 5.9a).

There was a positive correlation between shifting and the number of days in milk, for treatment 4 cows (r^2=49.6, $P<0.01$) (Table 5.9a). There was a tendency for treatment C cows to shift more as milk yield increased (P=0.06) (Table 5.12a).

There was a positive correlation between head swinging and lactation number for treatment C cows (r^2=23.1, P=0.03) (Table 5.10a).

The frequency of restlessness type behaviours increased with days in milk for treatment 4 cows (r^2=59.8, $P<0.01$) (Table 5.9a). There was also a positive correlation between this behaviour and milk yield for treatment C cows (P=0.01)

(Table 5.12a). There was a tendency for restlessness type behaviours to increase the closer the cows within treatment 4 were to parturition (P=0.07) (Table 5.13a).

Table 5.9a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with the number of days in milk in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Days in milk					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	0.97 -0.01	0.12 -0.36	**<0.01** **0.11**	-	-	0.02 27.7
Weight shifting	0.18 0.31	0.30 0.23	**0.04** **0.46**	-	-	0.02 28.4
Shifting	0.27 0.26	0.69 0.09	**<0.01** **0.61**	-	-	<0.01 49.6
Restlessness[1]	0.49 0.16	0.67 0.10	**<0.01** **0.63**	-	-	<0.01 59.8
Sniffing ground	*0.06* *0.44*	0.23 0.28	0.30 0.24	-	-	-
Grooming self	**0.02** **0.50**	0.75 -0.07	0.94 -0.02	-	-	-
Rubbing head against housing	**0.05** **0.43**	0.23 0.28	0.74 0.08	<0.01 46.2	-	-
Time spent rubbing head against housing	0.45 0.18	*0.06* *0.43*	*0.08* *0.41*	-	-	-
Body care	**0.01** **0.58**	0.65 0.11	0.66 0.10	0.01 31.2	-	-
Licking	**0.03** **0.49**	0.80 -0.06	0.68 -0.10	0.04 21.3	-	-
Stress	0.10 0.38	0.67 0.10	**0.01** **0.59**	-	-	<0.01 52.4
Time spent drinking	**0.02** **0.52**	0.18 0.31	0.55 -0.13	-	-	-
Sleeping	*0.08* *-0.40*	0.70 0.09	N/A	-	-	-
Walking	*0.09* *0.40*	0.60 -0.13	N/A	-	-	-

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$. [1]Relates to those behaviours that are indicative of this experience

Table 5.10a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with lactation number in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leqslant 0.05$) regressions

	Lactation number					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Head swinging	**0.03** **0.49**	0.61 -0.12	0.98 0.01	0.03 23.1	-	-
Time spent sniffing housing	0.67 -0.10	**0.01** **0.57**	0.17 0.32	-	0.02 25.8	-
Housing interaction	*0.06* *-0.43*	0.62 0.12	0.59 0.13	-	-	-

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leqslant 0.05$; Italicised P-values are used where $P < 0.10$

Table 5.11a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with body condition score in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leqslant 0.05$) regressions

	Body condition score					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	**0.01** **-0.58**	0.37 -0.21	0.79 0.06	0.02 26.8	-	-
Rubbing head against housing	0.95 0.02	*0.08* *0.40*	0.49 0.17	-	-	-
Butting	Not observed	0.52 -0.15	*0.08* *0.40*	-	-	-
Frustration[1]	0.87 0.04	0.63 -0.11	*0.07* *0.42*	-	-	-

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leqslant 0.05$; Italicised P-values are used where $P < 0.10$. [1]Relates to those behaviours that are indicative of this experience

There was a tendency for the cows in treatment C to sniff the ground more frequently the further they were into their lactation (P=0.06) (Table 5.9a).

Table 5.12a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with milk yield in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leqslant 0.05$) regressions

	Milk yield					
	Correlations (P-value; coefficient)			Regressions (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	*0.06* *0.42*	0.23 0.28	**0.04** **-0.47**	-	-	-
Shifting	*0.06* *0.42*	0.54 0.15	0.29 -0.25	-	-	-
Restlessness[1]	**0.01** **0.56**	0.26 0.26	0.20 -0.30	-	-	-

Bold P-values are used where $P \leqslant 0.05$; Italicised P-values are used where $P < 0.10$. [1]Relates to those behaviours that are indicative of this experience

Table 5.13a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with the number of days to parturition in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leqslant 0.05$) regressions

	Days to parturition					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Repositioning	N/A	**0.01** **-0.72**	0.38 0.28	-	-	-
Restlessness[1]	0.29 -0.35	0.40 -0.29	*0.07* *0.54*	-	-	-
Housing interaction	*0.07* *-0.57*	0.93 -0.02	0.95 0.02	-	-	-
Frustration[1]	*0.08* *0.55*	0.70 0.13	0.20 0.40	-	-	-
Walking	**0.01** **-0.73**	0.58 -0.19	N/A	0.04 40.7	-	-

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leqslant 0.05$; Italicised P-values are used where $P < 0.10$. [1]Relates to those behaviours that are indicative of this experience

The amount of time spent sniffing the housing increased with age for treatment 2 cows (r^2=25.8, P=0.02) (Table 5.10a). Self grooming was positively correlated with the number of days in milk for the treatment C cows (P=0.02) (Table 5.9a).

The cows in treatment C rubbed their heads against the housing more frequently the further they were into lactation (r^2=46.2, P<0.01) (Table 5.9a). There was a trend for a positive correlation between this behaviour and body condition score for treatment 2 cows (P=0.08) (Table 5.11a). Cows in treatments 2 and 4 tended to rub their heads against the housing for longer as the number of days in milk increased (P=0.06; P=0.08, respectively) (Table 5.9a). Body care type behaviours were positively correlated with the number of days in milk for treatment C cows (r^2=31.2, P=0.01) (Table 5.9a). There was a tendency for the older cows, within treatment C, to interacted with the housing less frequently (P=0.06) (Table 5.10a). Treatment C cows also tended to interact with the housing less, the closer they were to parturition (P=0.07) (Table 5.13a). Only treatment C cows were observed to increase licking type behaviours as the number of days in milk increased (r^2=21.3, P=0.04) (Table 5.9a). Stress type behaviours were positively correlated with the number of days in milk for treatment 4 cows (r^2=52.4, P<0.01) (Table 5.9a). There was also a tendency for butting to increase with increasing body condition score for treatment 4 cows (P=0.08) (Table 5.11a). In addition, there was a tendency for cows in treatment 4 to perform more frustration-related behaviours with increasing body condition score (P=0.07) (Table 5.11a). Frustration type behaviours also tended to increase the closer the treatment C cows were to parturition (P=0.08) (Table 5.13a). The amount of time spent drinking was positively correlated with the number of days in milk for treatment C cows (P=0.02) (Table 5.9a). Cows in treatment C also tended to sleep less frequently as the number of days in milk increased (P=0.08) (Table 5.9a). Cows in treatment C tended to walk more frequently as the number of days in milk increased (P=0.09) (Table 5.9a), but less frequently as the number of days to parturition increased (r^2=40.7, P=0.04) (Table 5.13a).

5.4.3.3a Behavioural correlations for continuous recordings

Self grooming and rubbing of the head against the housing were both significantly positively correlated with drinking for cows in treatments 2 and 4 (Table 5.14a). There was a trend towards a positive correlation between these same behaviours for treatment C cows (P=0.08; P=0.06, respectively). The performance of body care behaviours was also significantly positively correlated with drinking for cows in all treatments (Table 5.14a).

Table 5.14a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with drinking in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Drinking					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Grooming self	*0.08* *0.40*	**0.04** **0.46**	**0.03** **0.48**	-	-	0.04 22.1
Rubbing head against housing	*0.06* *0.43*	**<0.01** **0.65**	**0.05** **0.45**	-	-	-
Body care	**0.01** **0.54**	**<0.01** **0.61**	**0.02** **0.51**	-	-	0.02 26.0

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05; Italicised P-values are used where P<0.10

All three treatments significantly increased the frequency of the following behaviours as the time spent drinking increased: Self grooming, the total number of licks whilst self grooming, rubbing head against housing, licking and body care type behaviours (Table 5.15a). Leg raising behaviour decreased with increased drinking time for treatment 2 cows (P=0.01). Stress type behaviours were observed to increase with increasing time spent drinking, for treatment 4 cows (P=0.05). Weight shifting was

significantly positively correlated with drinking time for cows in treatments C and 4 (Table 5.15a).

Table 5.15a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with the time spent drinking in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Time spent drinking					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Grooming self	**0.06** **0.44**	**0.04** **0.47**	**0.02** **0.51**	0.02 28.6	0.02 28.2	-
Leg raising	0.32 -0.24	**0.01** **-0.56**	0.33 -0.23	-	-	-
Total number of licks whilst grooming self	**0.04** **0.46**	**0.04** **0.46**	**0.04** **0.46**	**<0.01** 24.0	**<0.01** 41.2	-
Licking	**<0.01** **0.69**	**<0.01** **0.66**	**<0.01** **0.68**	0.01 34.4	0.01 35.0	0.02 27.7
Rubbing head against housing	0.02 **0.51**	**<0.01** **0.78**	0.01 **0.58**	0.01 33.9	**<0.01** 62.5	-
Body care	**<0.01** **0.62**	**0.01** **0.56**	**0.01** **0.58**	**<0.01** 50.6	0.04 22.0	*0.06* *18.8*
Stress[1]	0.26 0.26	0.64 0.11	**0.05** **0.44**	-	-	-
Weight shifting	**0.02** **0.52**	0.10 0.38	**0.01** **0.58**	0.02 24.6	-	0.03 24.2

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$. [1]Relates to those behaviours that are indicative of this experience

As the frequency of frustration type behaviours increased, cows in treatment C significantly decreased the frequency of self grooming, the total number of licks whilst self grooming, licking behaviours, and body care type behaviours (Table 5.16a). There was a tendency for cows in treatment 4 cows to increase licking behaviours as they became more frustrated (P=0.06). Cows in treatment 4

significantly increased ground sniffing and sniffing behaviours as they became increasingly frustrated (Table 5.16a).

Cows in treatments C and 2 both self groomed more frequently as the number of times they interacted with the housing increased (r^2=27.2, P=0.02; r^2=35.9, P=0.01, respectively) (Table 5.17a). There was a negative correlation between frustration type behaviours and housing interaction, for cows in treatment C (r^2=26.3, P=0.02), but these variables were positively correlated for cows in treatment 4 (P=0.04).

Table 5.16a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with frustration type behaviours in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Frustration[1]					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Grooming self	**0.03** **-0.48**	0.35 -0.22	0.14 0.35	0.02 26.9	-	-
Total number of licks whilst grooming self	**0.01** **-0.60**	0.84 -0.05	0.39 0.21	0.01 29.2	-	-
Licking	**0.01** **-0.60**	0.89 -0.03	*0.06* *0.43*	0.01 33.6	-	-
Body care	**0.02** **-0.54**	0.12 -0.36	0.23 0.28	0.01 29.2	-	-
Sniffing	0.10 -0.38	0.84 -0.05	**0.02** **0.53**	-	-	-
Sniffing ground	0.12 -0.36	0.76 0.07	**0.04** **0.46**	-	-	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05; Italicised P-values are used where P<0.10. [1]Relates to those behaviours that are indicative of this experience

Housing interaction was positively correlated with the total number of licks whilst self grooming for cows in treatment C and 4 (P=0.05; P=0.02, respectively) (Table 5.17a). There was also a tendency for cows in treatment 2 to increase the number of licks whilst self grooming as they interacted more frequently with the housing (P=0.07). There was a positive correlation between housing interaction and restlessness type behaviours for cows in treatment 4 (r^2=26.0, P=0.02). Housing interaction was also positively correlated with stress type behaviours for this group (r^2=29.8, P=0.01) (Table 5.17a).

Treatment 4 cows groomed themselves more frequently the more they rubbed their heads against the housing (P=0.04) (Table 5.18a). There was also a non-significant correlation between these variables for cows in treatment 2 (P=0.06).

Table 5.17a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with housing interaction in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Housing interaction					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Grooming self	**0.02** **0.51**	**0.05** **0.44**	0.14 0.34	0.02 27.2	0.01 35.9	-
Frustration[1]	**<0.01** **-0.63**	0.56 -0.14	**0.04** **0.46**	0.02 26.3	-	-
Total number of licks whilst grooming self	**0.05** **0.44**	*0.07* *0.41*	**0.02** **0.53**	-	-	-
Restlessness[1]	0.98 -0.01	0.70 -0.10	**0.04** **0.47**	-	-	0.02 26.0
Stress[1]	0.39 0.20	0.70 0.09	**0.03** **0.49**	-	-	0.01 29.8

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05; Italicised P-values are used where P<0.10. [1]Relates to those behaviours that are indicative of this experience

Table 5.18a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with self grooming in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leqslant 0.05$) regressions

	Grooming self					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Rubbing head Against housing	0.27 0.26	*0.06* *0.43*	**0.04** **0.46**	-	-	-
Shifting	*0.06* *0.42*	**0.04** **0.46**	0.31 0.24	-	-	-

Bold P-values are used where $P \leqslant 0.05$; Italicised P-values are used where $P < 0.10$

Weight shifting and shifting were both significantly negatively correlated with head swinging for cows in treatments C and 2 (Table 5.19a). There was a positive correlation between butting and head swinging for the treatment 2 cows (P=0.05) (Table 5.19a).

Table 5.19a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with head swinging, in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leqslant 0.05$) regressions

	Head swinging					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Butting	N/A	**0.05** **0.44**	0.80 0.06	-	-	-
Shifting	**0.03** **-0.49**	**0.01** **-0.60**	0.31 0.24	0.04 21.1	0.02 26.7	-
Body care	0.42 -0.19	**0.03** **-0.50**	0.40 0.20	-	-	-
Weight shifting	**0.03** **-0.48**	**<0.01** **-0.66**	0.41 0.19	-	0.01 31.5	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leqslant 0.05$

Table 5.20a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with leg raising, in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Leg raising					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Head swinging	0.80 -0.06	0.52 0.16	**0.03** **0.49**	-	-	-
Repositioning	N/A	0.36 -0.22	**0.03** **-0.48**	-	-	-

Bold P-values are used where $P \leq 0.05$

Leg raising was positively correlated with head swinging for cows in treatment 4 (P=0.03) and negatively correlated with repositioning (P=0.03) (Table 5.20a).

There was a positive correlation between rubbing of the head against the housing and the number of licks whilst self grooming for cows in treatments 2 and 4 (P=0.03 and r^2=55.0, $P < 0.01$, respectively) (Table 5.21a).

Licking behaviours were significantly positively correlated with drinking and rubbing of the head against the housing for cows in all three treatments (Table 5.22a). Ground sniffing increased with increasing frequency of licking behaviours for cows in treatment C (r^2=26.7, P=0.02). Cows in treatments C and 2 were observed weight shifting significantly more frequently as the frequency of licking behaviours increased (Table 5.22a).

Table 5.21a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with the number of licks whilst self grooming in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Number of licks whilst grooming self					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Rubbing head against housing	0.70 0.09	**0.03** **0.49**	**<0.01** **0.66**	-	<0.01 55.0	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$

Table 5.22a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with licking behaviours in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Licking behaviours					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Drinking	**<0.01** **0.85**	**<0.01** **0.77**	**<0.01** **0.83**	<0.01 65.0	<0.01 55.6	<0.01 62.7
Rubbing head against housing	**0.04** **0.47**	**<0.01** **0.64**	**0.05** **0.44**	-	0.01 36.8	-
Sniffing ground	**0.05** **0.44**	0.23 0.28	0.19 0.30	0.02 26.7	-	-
Weight shifting	**0.01** **0.60**	**0.02** **0.52**	0.41 0.19	0.01 34.3	-	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$

The time spent rubbing head against the housing was significantly positively correlated with drinking for cows in treatments 2 and 4 (Table 5.23a), whilst there was a non-significant positive correlation between these variables for treatment C cows ($P=0.09$). The longer the treatment 2 cows spent rubbing their heads against the housing the more frequently they groomed themselves ($r^2=47.8$, $P<0.01$). Time spent

rubbing heads against housing was significantly positively correlated with licking behaviours, time spent drinking, and the total number of licks whilst self grooming for cows in treatments 2 and 4.

Table 5.23a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with the time spent rubbing head against the housing, in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Time spent rubbing head against housing					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Drinking	*0.09* *0.39*	**0.02** **0.53**	**0.03** **0.49**	-	-	0.02 26.0
Grooming self	0.69 0.09	**0.02** **0.52**	0.10 0.38	-	<0.01 47.8	-
Total number of licks whilst grooming self	0.79 -0.06	**0.05** **0.44**	**0.01** **0.55**	-	-	-
Licking	0.20 0.30	**0.01** **0.61**	**0.05** **0.44**	-	-	0.05 20.1
Time spent drinking	0.17 0.32	**0.01** **0.55**	**0.01** **0.59**	-	0.04 21.6	<0.01 47.7

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$

Self grooming, the total number of licks whilst self grooming, and weight shifting were all significantly positively correlated with sniffing housing for cows in treatment C (Table 5.24a). Restlessness and stress type behaviours were significantly positively correlated with sniffing housing for cows in treatment 4.

Both self grooming and weight shifting significantly increased as ground sniffing increased in all treatments (Table 5.25a). Cows in treatments 2 and 4 significantly

increased restlessness-related and shifting behaviours the more they sniffed the ground. Stress type behaviours were positively correlated with ground sniffing for cows in treatments 2 and 4 (r^2=41.2, P<0.01; P<0.01, respectively) (Table 5.25a).

Table 5.24a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with sniffing housing in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Sniffing housing					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Grooming self	**0.03** **0.49**	0.53 0.15	0.78 0.07	0.01 32.7	-	-
Total number of licks whilst grooming self	**0.01** **0.59**	0.83 0.05	0.48 0.17	0.03 22.6	-	-
Restlessness[1]	0.66 -0.11	0.76 -0.07	**0.01** **0.55**	-	-	0.04 20.9
Stress[1]	0.52 0.16	0.95 -0.02	**0.03** **0.50**	-	-	0.05 19.6
Weight shifting	**0.04** **0.47**	0.76 0.07	*0.08* *0.40*	0.05 20.2	-	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05; Italicised P-values are used where P<0.10. [1]Relates to those behaviours that are indicative of this experience

Sniffing behaviours were positively correlated with self grooming for cows in treatments C and 2 (r^2=33.8, P=0.01; r^2=34.4, P=0.01, respectively) (Table 5.26a). There was a positive correlation between the total number of licks whilst self grooming and sniffing for cows in treatment C (r^2=20.1, P=0.05). Restlessness-related, shifting and stress type behaviours all increased significantly as sniffing increased, in treatment 2 and 4 cows. Weight shifting was significantly positively correlated with sniffing for cows in all treatments (Table 5.26a).

Table 5.25a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with sniffing the ground, in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Sniffing ground					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Grooming self	**0.02** **0.54**	**0.03** **0.48**	**0.03** **0.49**	0.02 25.9	0.01 29.3	<0.01 45.9
Restlessness[1]	0.46 0.18	**0.01** **0.54**	**<0.01** **0.64**	-	0.02 25.8	-
Shifting	0.01 0.38	**0.02** **0.52**	**<0.01** **0.67**	-	0.02 25.6	-
Stress[1]	*0.09* *0.40*	**<0.01** **0.62**	**<0.01** **0.74**	-	<0.01 41.2	-
Weight shifting	**<0.01** **0.78**	**0.01** **0.56**	**<0.01** **0.64**	<0.01 69.4	0.01 30.6	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$. [1]Relates to those behaviours that are indicative of this experience

Table 5.26a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with sniffing behaviours in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Sniffing behaviours					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Grooming self	**0.01** **0.58**	**0.01** **0.58**	0.12 0.36	0.01 33.8	0.01 34.4	-
Total number of licks whilst grooming self	**0.05** **0.54**	*0.09* *0.39*	0.13 0.35	0.05 20.1	-	-
Restlessness[1]	0.74 0.08	**0.04** **0.46**	**0.01** **0.58**	-	0.04 20.6	-
Shifting	0.25 0.27	**0.05** **0.44**	**0.02** **0.44**	-	0.04 22.0	-
Stress[1]	0.14 0.34	**0.01** **0.57**	**<0.01** **0.63**	-	0.01 35.5	0.01 30.7
Weight shifting	**<0.01** **0.74**	**0.01** **0.58**	**0.02** **0.52**	<0.01 65.5	0.02 28.1	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$. [1]Relates to those behaviours that are indicative of this experience

Table 5.27a The significance and coefficient for the average frequency, or duration, of each behaviour, recorded over the entire deprivation period, which was significantly correlated with walking, in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Walking					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	0.34 -0.22	**0.01** **0.59**	N/A	-	<0.01 71.1	-
Restlessness[1]	0.67 0.10	**0.05** **0.45**	N/A	-	0.03 24.1	-
Shifting	0.13 0.35	**0.02** **0.51**	N/A	-	0.02 26.8	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$. [1]Relates to those behaviours that are indicative of this experience

Table 5.28a The significance and coefficient for the average frequency, or duration, of each behaviour, recorded over the entire deprivation period, which was significantly correlated with weight shifting, in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Weight shifting					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Repositioning	N/A	**0.02** **0.52**	0.31 0.35	-	0.03 24.4	-
Body care	**0.04** **0.47**	**0.01** **0.60**	**0.05** **0.45**	-	-	-
Time spent sniffing housing	**0.05** **0.44**	0.94 0.02	0.38 0.21	0.05 20.4	-	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$

Leg raising, restlessness-related, and shifting behaviours for the treatment 2 cows were all significantly positively correlated with walking (Table 5.27a).

Cows in all treatments significantly increased the frequency of body care type behaviours the more they shifted their weight (Table 5.28a). There was a positive correlation between repositioning and weight shifting for treatment 2 cows (r^2=24.4, P=0.03). Treatment C cows increased the amount of time they spent sniffing the housing, the more frequently they shifted their weight (r^2=20.4, P=0.05) (Table 5.28a).

5.4.3.4a Instantaneous recordings

Each sub-table (1 to 8) in Table 5.29a shows the differences between values for each hour (by row) and for each treatment (by column), for a specific behaviour.

There was no difference between hours in the amount of time the treatment C cows spent feeding (P=0.54) (Table 5.29a1). Treatment 2 cows spent the longest period of time feeding in the first hour of actual deprivation, which was only significantly different from the second hour, where they spent the least amount of time feeding. There was no significant difference between any of the other hours for this group. Treatment 4 cows spent longer feeding in their first hour of deprivation compared with hours two to four (P=0.02).

There was no significant difference between cows in treatments C and 2 for the time spent feeding in the first two hours of the deprivation period (Table 5.29a1). Treatment 4 cows spent 11.5 and 10.0 min longer feeding than cows in treatments C and 2 in the first and second hour, respectively (P=0.02 and P=0.01, respectively). There was no treatment effect on feeding time in the third hour (P=0.11). Feeding time increased, between treatments, with increasing deprivation time in the fourth

hour (P=0.01). Over the entire deprivation period, treatment 4 cows spent significantly longer feeding in comparison to cows in the other two treatments ($P < 0.01$), which were not significantly different. The proportion of time spent in this activity over the deprivation period for cows in treatments C, 2 and 4 was 24.6, 30.3 and 37.4%, respectively.

Cows in treatment C spent longer standing in the first hour than in any other hour (P=0.05) (Table 5.29a2). The time spent standing in the proceeding three hours remained approximately the same for this group. Treatment 2 cows spent significantly longer standing in the last two hours, which were not significantly different, in comparison the first two hours, which were not significantly different. Treatment 4 cows reduced the amount of time spent standing per hour over the course of the deprivation period. However, there was only a significant difference between the first and fourth hour in standing time for cows in this treatment.

Table 5.29a The average time (min h^{-1}) that each behaviour was performed per hour, and for all four hours, during the deprivation period, for all of the experimental cows in the control (C), 2 h (2) and 4 h (4) treatments

1. Feeding	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours
C(m)	b 17.3 15.0	b 10.0	15.0	10.0	-	0.54	b 57.5
2(m)	b 17.3 a,b	10.8 b b 10.0	24.3 a 25.0	18.3 a,b 15.0	3.51	**<0.01**	b 70.5
4(m)	a 28.8 30.0	a 20.0	20.0	22.5	-	**0.02**	a 87.5
SED ±	4.49	-	-	-			7.06
P-value between treatments	**0.02**	**0.01**	0.11	**0.01**			**<0.01**

(m)Mean values on top row

2. Standing	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours
C	b 15.0	5.0	b 7.5	5.0	-	**0.05**	c 33.8
2[(m)]	14.5 [b] b 15.0	11.8 [b] 12.5	22.8 [a] a 20.0	23.3 [a] 20.0	3.09	**<0.01**	b 72.3
4[(m)¶]	24.6 [a] a 27.5	24.5 [a,b] 27.5	20.1 [a,b] a 20.0	16.3 [b] 15.0	3.26	**0.03**	a 93.8
SED ±	(2&4)[¶] 3.35	(2&4)[¶] 3.07	(2&4)[¶] 2.85	(2&4)[¶] 3.44			6.66
P-value between treatments	(2&4)[¶] **<0.01** **<0.01**	(2&4)[¶] **<0.01** **<0.01**	(2&4)[¶] **0.73** **<0.01**	(2&4)[¶] **0.10** **<0.01**			**<0.01**

[m]Mean values on top row; [¶]Used back transformed data to calculate mean values. [1]Numbers in parenthesis represent the hours/treatments to which the following P-value/SED refers

3. Lying	Hour 1	2	3	4	P-value between hours	Mean time for all hours
C[(m)]	5.0	13.8 12.5	10.0	15.0	0.50	a 53.5
2[(m)]	5.0 [a]	14.9 10.0 [a]	-	-	0.658	b 25.8
4	-	-	-	-	-	-
SED ±	-	4.04	-	-		5.43
P-value between treatments	0.99	0.78	-	-		**<0.01**

[m]Mean values on top row

4. Standing ruminating	Hour 1	2	3	4	SED ±	P-value between hours	Median time for all hours
C	b 0-1 [a]	c 0 [b]	c 0 [b]	b 0-1 [a]	-	-	2.5
2[(m)]	b 0-1 [a]	b 0-1 [a]	b 11.8 10.0 [b]	a 18.0 17.5 [b]	(3&4)[¶] 3.53	(3&4) 0.09	32.5
4[(m)]	a 1.0	a 12.5	a 20.8 20.0	a 21.8 22.5	(3&4)[¶] 3.15	(3&4) 0.75 **<0.01**	52.5
SED ±	-	-	(2&4)[¶] 3.29	(2&4)[¶] 3.37			-
P-value between treatments	-	-	(2&4)[¶] **0.01**	(2&4)[¶] 0.27			**<0.01**

[m]Mean values on top row. [¶]Numbers in parenthesis represent the hours/treatments to which the following P-value/SED refers

5. Lying ruminating	Hour 1	2	3	4	P-value between hours	Mean time for all hours
C[(m)]	7.5	25.0 27.5	25.5	22.5	0.09	[b] 86.3
2[(m)]	10.0[a]	19.3 20.0[a]	-	-	0.12	[a] 32.0
4	-	-	-	-	-	-
SED ±	-	4.38	-	-		6.39
P-value between treatments	0.79	0.20	-	-		**<0.01**

[m]Mean values on top row

6. All standing behaviours*	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours
C[(m)]	[b] 15.8 15.0	5.0	[b] 10.9 10.0	7.5	(1&3)[1] 3.54	(1&3)[1] 0.13 0.14	[c] 91.3[c]
2[(m)]	[b] 19.0[b]	17.0[b] 15.0	[a] 34.5[a]	41.3[a] 42.5	4.01	**<0.01**	[b] 142.8[b]
4[(m)]	[a] 31.3[b] 30.0	40.0	[a] 41.5 40.0	37.5	(1&3)[1] 3.59	(1&3)[1] <0.01 **0.02**	[a] 181.3[a]
SED ±	(C&4)[1] 4.17 4.37	-	(C&4)[1] 2.83 3.49	-			6.68
P-value between treatments	(C&4)[1] **<0.01** **<0.01**	**<0.01**	(C&4)[1] **<0.01** **<0.01**	**<0.01**			**<0.01**

[m]Mean values on top row; *standing + ruminating standing. [1]Numbers/letter in parenthesis represent the hours/treatments to which the following P-value/SED refers

7. All lying behaviours*	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours
C[(m)]	27.0	41.0	33.3	38.5	5.60	0.07	[a] 139.8
2[(m)]	23.8	32.3	-	-	6.32	0.19	[b] 57.8
4	-	-	-	-	-	-	-
SED ±	6.54	5.70	-	-			8.15
P-value between treatments	0.62	0.13	-	-			**<0.01**

[m]Mean values; *lying + ruminating lying

8. All standing behaviours & Feeding*	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours
C[(m)]	33.0	19.0	26.8	21.5	5.60	0.07	$_{b}$ 100.3
2[(m)]	36.3 ^{a}	27.8 ^{a}	-	-	6.32	0.19	$_{a}$ 182.3
4	-	-	-	-	-	-	-
SED ±	6.54	5.70	-	-			8.15
P-value between treatments	0.62	0.13	-	-			**< 0.01**

[m]Mean values; *standing + ruminating standing + feeding

Key:

- Row values with different superscripts are significantly different ($P \leqslant 0.05$).
- Treatment values with different subscripts are significantly different ($P \leqslant 0.05$).
- Where letters to indicate differences between values are absent, but a significant probability value has been shown, a non-parametric test was performed and therefore no post-hoc test of paired means could be carried out. In this case, a value judgement may be made about the data.
- All values are medians unless otherwise stated.
- The symbol '-' in the treatment/hour box indicates that the cows for that treatment were unable to perform the behaviour in that hour.
- '0-1' denotes that the median is zero, but the 1-Sample Sign test indicates that the true median lies between zero and one.
- Bold P-values are used where $P \leqslant 0.05$.

Cows in treatment 4 spent longer (median 17.5 min) standing in the first hour than treatment C and 2 cows ($P < 0.01$), which were not significantly different to each other (Table 5.29a2). Standing time increased, between treatments, with increasing deprivation time in the second hour ($P < 0.01$). There was no significant difference in

standing time between cows in treatments 2 and 4 in the third hour, but cows in either treatment stood longer than cows in treatment C ($P<0.01$). In the fourth hour, cows in treatment 2 spent the most time standing and treatment C cows spent the least time standing ($P<0.01$). Over the entire four hour period, the time spent standing increased, between treatments, with increasing deprivation time ($P<0.01$). The proportion of time spent in this activity over the deprivation period for cows in treatments C, 2 and 4 was 14.5, 31.0 and 40.1%, respectively.

Lying time did not change over time for cows in treatments C and 2 ($P=0.50$; $P=0.66$, respectively) (Table 5.29a3). There was no difference in lying time between cows in treatments C and 2 in the first or second hour ($P=0.99$; $P=0.78$, respectively) (Table 5.29a3). Over the entire deprivation period, treatment C cows spent a mean time of approximately 28 min longer lying than cows in treatment 2 (22.9 versus 11.1%, respectively) ($P<0.01$).

The median frequency of standing ruminating was low for cows in treatment C and not clearly affected by deprivation length (Table 5.29a4). Treatment 2 cows spent significantly longer standing ruminating in the last two hours (median 10.0 and 17.5 min, respectively), which were not significantly different, in comparison to the first two hours (median 0-1 for both hours), which were not significantly different. This activity increased with time for the treatment 4 cows ($P<0.01$).

The incidence of standing ruminating was low for cows in all treatments in the first hour and was not clearly affected by treatment (Table 5.29a4). The time spent standing ruminating significantly increased, between treatments, with increasing

deprivation time in the second and third hour. There was no significant difference between cows in treatments 2 and 4 in time spent standing ruminating in the fourth hour, but they both spent significantly longer performing this behaviour than treatment C cows. Over the entire deprivation period, the time spent standing ruminating increased, between treatments, with increasing deprivation time ($P < 0.01$). The proportion of time spent in this activity over the deprivation period for cows in treatments C, 2 and 4 was 1.1, 13.9 and 22.5%, respectively.

There was no difference between hours for lying ruminating in treatment C or 2 cows ($P=0.09$; $P=0.12$, respectively) (Table 5.29a5). There was no significant difference between cows in treatments C and 2 for lying ruminating in either the first or the second hour (Table 5.29a5). Over the entire deprivation period, treatment C cows spent a mean time of approximately 54 min longer than treatment 2 cows ruminating whilst lying (36.9 versus 13.7%, respectively) ($P < 0.01$).

There were no differences between hours in the time spent in standing behaviours, *i.e.* standing and ruminating standing combined, for treatment C cows ($P=0.14$) (Table 5.29a6). Cows in treatment 2 spent longer in these behaviours in the third and fourth hour, which were not significantly different, in comparison to the first and second hour, which were not significantly different ($P < 0.01$). Cows in treatment 4 increased the amount of time in standing behaviours by a median of 10.0 min in the second and also in the third hour, and 7.5 min in the fourth hour, in comparison to the first hour ($P=0.02$).

Standing behaviours increased between treatments with increasing deprivation time in all hours, except the fourth, and over the entire deprivation period (Table 5.29a6). In the fourth hour, treatment 2 cows spent the longest time in standing behaviours and treatment C cows the least. In the first hour, there was no significant difference between cows in treatments C and 2. In the third hour, there was no significant difference between treatment 2 and 4 cows.

There were no significant differences between hours in the time spent in total lying behaviours, *i.e.* lying and ruminating lying combined, for treatment C cows (Table 5.29a7). There was no difference between the first and the second hour for lying behaviours in treatment 2 cows (P=0.19).

There was no difference between cows in treatments 2 and 4 in lying behaviours for the first or second hour (P=0.62; P=0.13, respectively) (Table 5.29a7). Over the entire deprivation period, treatment C cows spent a mean time of 82 min longer than treatment 2 cows in lying behaviours ($P < 0.01$). In total, cows in treatment C spent approximately 140 min (2h 19 min) lying over the four hour period.

There was no significant difference between hours for treatment C cows in the total amount of time spent in standing behaviours and feeding, when combined (Table 5.29a8). There was no difference between the first and second hour for cows in treatment 2 in these behaviours (P=0.19). As these were the only behaviours that could be performed by cows in treatments 2 and 4 during deprivation, this combined behaviour was not analysed.

There was no difference between cows in treatments C and 2 in the total amount of time spent in standing behaviours and feeding, when combined, in the first or second hour (P=0.62; P=0.13, respectively) (Table 5.29a8). Over the entire deprivation period, treatment 2 cows spent a mean time of 82 min longer in comparison to treatment C cows in these behaviours ($P < 0.01$).

Over the entire deprivation period, cows in treatment C spent most of their time lying ruminating (36.9%), then feeding (24.6%), lying (22.9%), standing (14.5%) and the least time standing ruminating (1.1%). In contrast, cows in treatments 2 and 4 spent approximately equal amounts of time feeding and standing (Treatment 2: 30.3 and 31.0%, respectively. Treatment 4: 37.4 and 40.1%, respectively). Cows in treatment 2 spent approximately equal amounts of time lying, lying ruminating and standing ruminating (11.1, 13.7 and 13.9%, respectively), whilst treatment 4 cows spent the rest of their time standing ruminating only (22.5%).

5.4.3.5a Behavioural versus production variable correlations for instantaneous recordings

The time spent feeding decreased with increasing milk yield for treatment 2 cows (r^2=21.4, P=0.04) (Table 5.30a). Cows in treatment 4 spent less time standing as milk yield increased (r^2=36.5, P=0.01) (Table 5.30a). There was a tendency for standing time to increase with age in treatment 2 and 4 cows (P=0.06; P=0.06, respectively).

Standing ruminating and milk yield were positively correlated for treatment 4 cows (r^2=42.8, $P < 0.01$) (Table 5.30a). There was a positive relationship between standing

ruminating and the number of days to parturition for cows in treatment 2 (r^2=69.1, $P < 0.01$).

Table 5.30a The significance and coefficient for the average duration of each behaviour, recorded over the entire deprivation period, which was significantly correlated with the milk yield, number of days to parturition, and lactation number in at least one of the control (C), 2 h (2) and 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Correlation (P-value; coefficient)			r^2-value		
	Milk yield					
Behaviour	C	2	4	C	2	4
Feeding	0.26 0.26	**0.04** **-0.46**	0.78 0.07	-	21.4	-
Standing	0.67 -0.10	0.65 -0.11	**0.01** **-0.60**	-	-	36.5
Standing ruminating	0.42 -0.19	0.14 0.34	**<0.01** **0.65**	-	-	42.8
All standing	0.46 0.17	**0.03** **-0.49**	**<0.01** **-0.65**	-	23.9	42.8
	Days to parturition					
Standing ruminating	0.36 -0.31	**<0.01** **0.83**	0.11 0.48	-	69.1	-
	Lactation number					
lying ruminating	0.12 -0.36	**0.01** **0.54**	N/A	-	29.5	-
Standing	0.39 -0.20	*0.06* *0.43*	*0.06* *0.43*	-	-	-

Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$

There was a negative correlation between standing behaviours and milk yield for cows in treatments 2 and 4 (r^2=23.9, P=0.03; r^2=42.8, $P < 0.01$, respectively) (Table 5.30a).

The amount of time spent lying ruminating increased with age in treatment 2 cows (r^2=29.5, P=0.01) (Table 5.30a).

5.4.3.6a Behavioural correlations for instantaneous recordings

Ruminating lying was negatively correlated with feeding for cows in treatment 2 (r^2=20.8, P=0.04). The time spent standing decreased as feeding time increased for cows in treatments C and 4 (r^2=21.6, P=0.04; r^2=43.4, P<0.01, respectively). Other behaviours, when correlated, were significant but this was due to the finite recording method employed. For example, the amount of time spent standing was significantly negatively correlated with time spent lying. For this reason, such correlations are not reported.

5.4.4a Lying position and changes in posture post-deprivation

No changes in posture were observed during the first 50 min post-deprivation for any of the experimental cows observed lying in any treatment. All of the experimental cows, for each treatment, observed lying during this period preferred to lie laterally, except one treatment 2 cow, which adopted a sternum lying position.

There was no strong evidence to suggest that lying side and length of deprivation were related (P=0.55) (Table 5.31a). Nearly all of the cows (95%) in treatment 4 chose to lie within the first 50 min following the deprivation period, compared with 85% for treatment 2 and 55% for treatment C.

Table 5.31a The number of experimental cows that were observed lying on their left or right side during the first hour post-deprivation, for the control (C), 2 h (2) and 4 h (4) treatments

Lying side	C	2	4
Left	5 (45%)	11 (65%)	12 (63%)
Right	6	6	7
Total	11	17	19

5.4.5a Post-deprivation video observations

Within the first eight hours post-deprivation (14.25 to 22.20 h) the time spent feeding decreased, between treatments, with increasing deprivation time (Table 5.32a).

Table 5.32a The mean time (min) spent feeding every eight hours, for the first 40 hours immediately following lying deprivation, for the whole period (hours 1-40) and for the whole period including the deprivation period (hours-4-40), for all the experimental animals in the control (C), 2 h (2) and 4 h (4) treatments

Hour, post-deprivation. (actual time)	Treatment C	2	4	SED ±	P-value
1 – 8 (1425-2220)	97.3 [a]	71.8 [b]	62.3 [b]	6.67	**< 0.01**
9-16[¶] (2225-0620)	14.4 [b] (1.158)	16.7 [a,b] (1.223)	26.6 [a] (1.425)	(0.0501)	**0.01**
17-24 (0625-1420)	85.5	92.5	76.8	6.36	0.22
25-32 (1425-2220)	102.5	102.3	87.5	6.00	0.14
33-40[¶] (2225-0625)	22.6 (1.355)	26.9 (1.429)	24.5 (1.390)	(0.0643)	0.76
1-41 (1425-0625)	325.3 [a]	310.0 [a,b]	271.8 [b]	14.38	**0.03**
-4-41 (1025-0625)	382.8	380.5	359.3	16.50	0.54

[¶] Used back transformed data to calculate values. Transformed values in parenthesis. Bold P-values are used where P≤0.05. Values with different superscripts are significantly different (P≤0.05)

During this period treatment C cows spent 25.5 and 35.0 min longer feeding than cows in treatments 2 and 4, respectively ($P < 0.01$). Cows in treatments 2 and 4 were not significantly different from each other for this activity during this time. The proportion of time spent feeding during this period for cows in treatments C, 2 and 4 was 23.7, 17.5, and 15.2%, respectively. From Fig. 5.2a it appears that the greatest reduction in feeding time between the cows in the deprived treatments and the treatment C cows took place in the first hour post-deprivation (14.25 h) and the first hour post-milking (17.25 h). From 18.25 to 21.25 h, except 20.25 h, treatment 4 cows

spent less time feeding than cows in treatments C and 2, which appeared to feed for approximately the same amount of time (Fig 5.2a). In the second eight hour period (22.25 to 06.20 h), the treatment C cows spent significantly less time feeding (3.0%) in comparison to treatment 4 cows (5.5%) (P=0.01) (Table 5.32a). There was no significant difference between cows in treatment 2, which spent 3.5% of their time feeding within this period, and the cows in the other two treatments. There was no significant difference in feeding time between treatments for any of the remaining eight hour periods.

Over the entire 41 h period, cows in treatment 4 spent 54 and 39 min less time feeding in comparison to cows in treatments C and 2, respectively (Table 5.32a, hours 1 to 41). However, cows in treatment 4 were only significantly different from treatment C cows for this activity (P=0.03). There was no significant difference in feeding time between cows in treatments C and 2. The proportion of time spent feeding over the 41 h period for cows in treatments C, 2 and 4 was 15.9, 15.8 and 14.9%, respectively. There was no difference in feeding time between any of the treatments when the data from the deprivation period was included (P=0.54) (Table 5.32a, hours - 4 to 41).

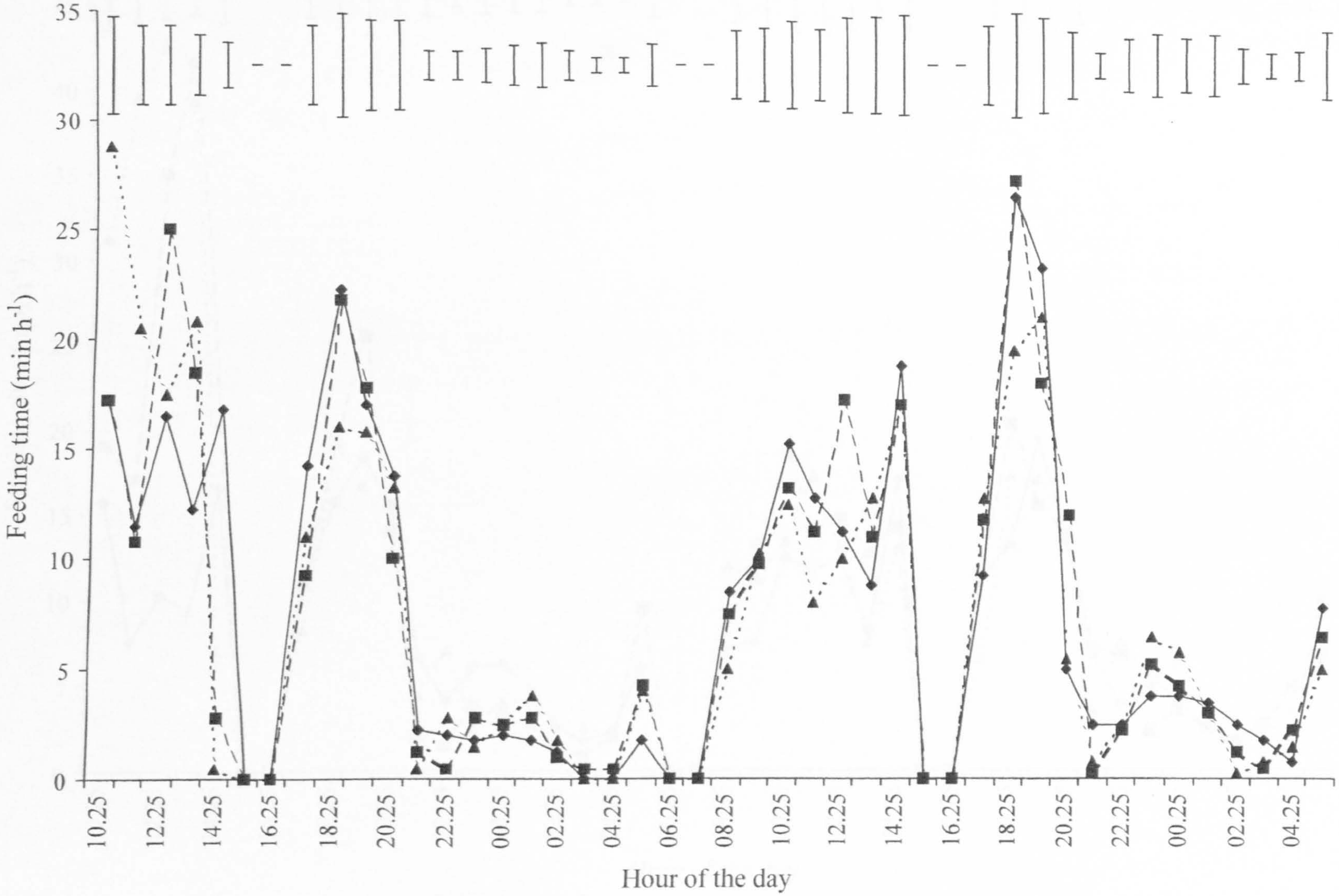


Fig 5.2a The mean time (min h^{-1}) spent feeding for all the experimental animals in the control ——◆—— , 2 h deprivation – – –■– – – , and 4 h deprivation ----▲---- treatments (I = SED bars)

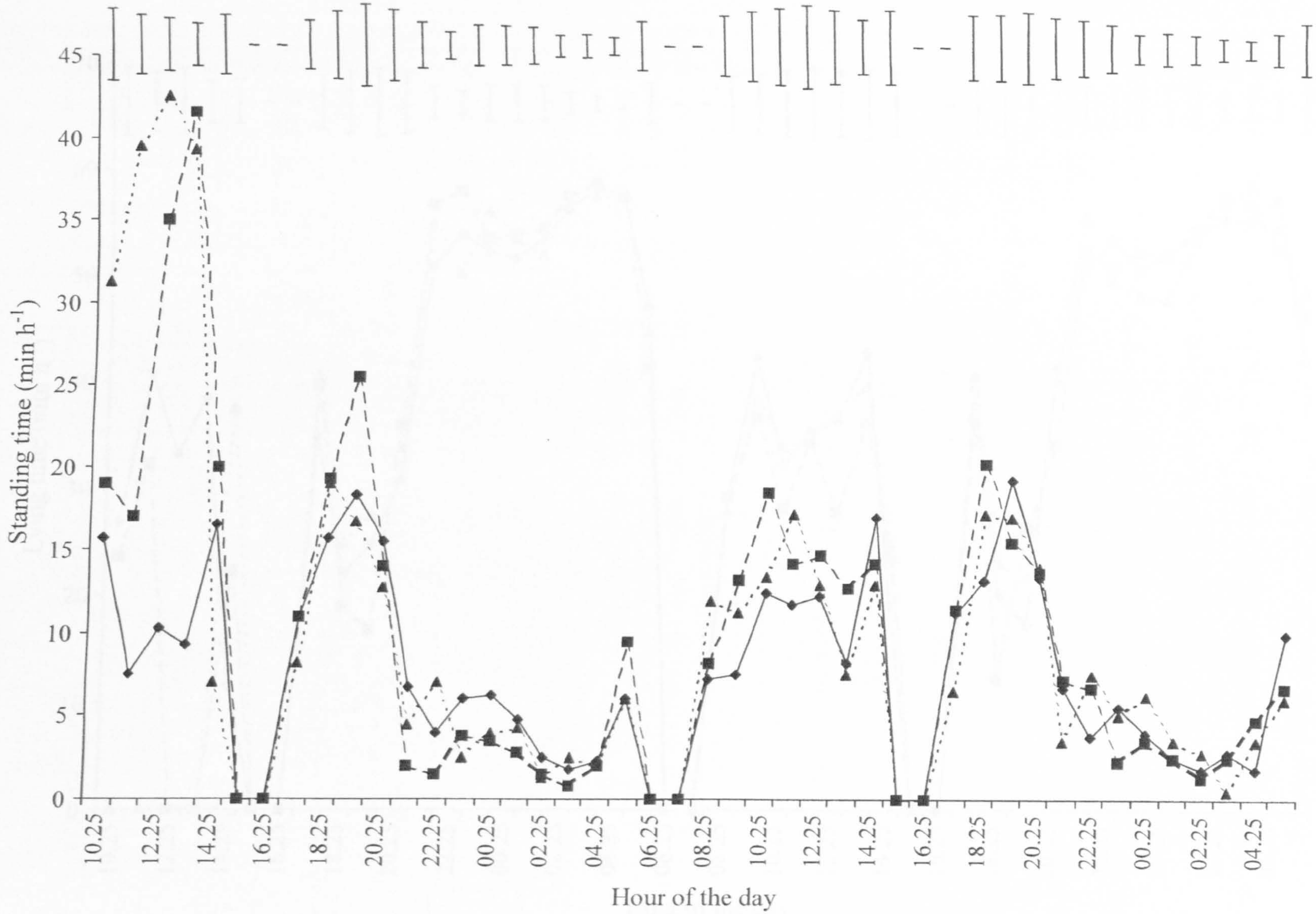


Fig 5.3a The mean time (min h^{-1}) spent standing only for all the experimental animals in the control ——◆—— , 2 h deprivation ––■––– , and 4 h deprivation ----▲---- treatments (I = SED bars)

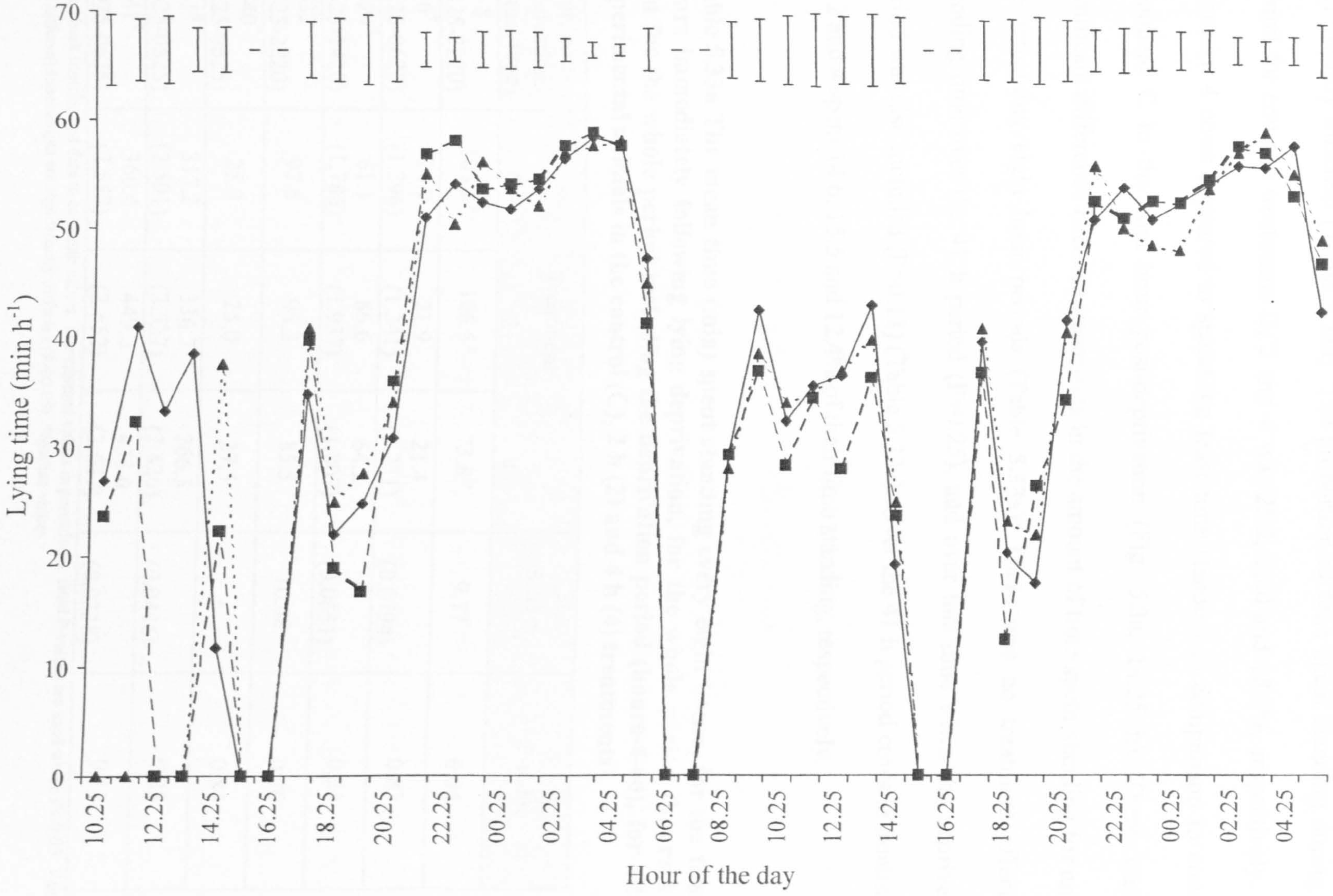


Fig 5.4a The mean time (min h^{-1}) spent lying only for all the experimental animals in the control ——◆—— , 2 h deprivation ——■—— , and 4 h deprivation ----▲---- treatments (I = SED bars)

In the first eight hours post-deprivation treatment 4 cows spent significantly less time standing (c. 30 min) in comparison to cows in treatments C and 2, which were not significantly different (Table 5.33a). The proportion of time spent standing during this period for cows in treatments C, 2 and 4 was 25.2, 26.0 and 18.0%, respectively. The treatment 4 cows appeared to spend the least time standing in comparison to cows in treatment C in the first hour post-deprivation (Fig. 5.3a, 14.25 h). There was no significant difference between treatments in the amount of time spent standing for any of the remaining eight hour periods (Table 5.33a). There was no treatment effect on standing time over the 41 h period (P=0.25), and over this time when the deprivation period was also included (P=0.11) (Table 5.33a). Over the 41 h period cows in treatments C, 2 and 4 spent 14.6, 15.5 and 12.4% of their time standing, respectively.

Table 5.33a The mean time (min) spent standing every eight hours, for the first 40 hours immediately following lying deprivation, for the whole period (hours 1-40) and for the whole period including the deprivation period (hours-4-40), for all the experimental animals in the control (C), 2 h (2) and 4 h (4) treatments

Hour, post-deprivation. (actual time)	Treatment C	2	4	SED ±	P-value
1 – 8 (1425-2220)	103.3 [a]	106.5 [a]	73.8 [b]	9.77	**0.04**
9-16¶ (2225-0620)	24.9 (1.396)	21.9 (1.341)	21.4 (1.331)	(0.0794)	0.83
17-24¶ (0625-1420)	61.1 (1.786)	86.6 (1.937)	64.2 (1.807)	(0.0651)	0.21
25-32 (1425-2220)	97.5	95.3	85.5	10.68	0.70
33-40* (2225-0625)	25.0	25.0	27.5	-	0.84
1-41¶ (1425-0625)	317.2 (2.501)	336.7 (2.527)	266.5 (2.426)	(0.0446)	0.25
-4-41¶ (1025-0625)	360.4 (2.557)	449.2 (2.652)	433.0 (2.637)	(0.0338)	0.11

¶ Used back transformed data to calculate values. Transformed values in parenthesis. Bold P-values are used where P≤0.05. Values with different superscripts are significantly different (P≤0.05). *Median values.

There was only a significant difference between treatments in lying time for the first eight hours post-deprivation (Table 5.34a). Lying time increased, between treatments, with increasing deprivation time ($P<0.01$). Cows in treatments 2 and 4 spent approximately 22 and 65 min, respectively, longer lying than treatment C cows in this period. However, there was no significant difference between cows in treatment C and cows in treatment 2 for this behaviour during this time. The proportion of time spent lying during this period was 51.1, 56.5 and 66.1% for cows in treatments C, 2 and 4, respectively. The greatest difference in mean lying time between the cows in treatments C and 4, during the first eight hour period, took place in the first hour following deprivation (14.25 h) and the first hour post milking (17.25 h) (Fig. 5.4a). Between the 17^{th} and 24^{th} h (06.25 to 14.20 h) following deprivation, there was a tendency for the treatment 2 cows to spend less time lying than cows in treatments C and 4 ($P=0.07$) (Table 5.34a).

Although cows in treatment 4 spent approximately 86 min more time lying than cows in treatments C and 2 over the entire 41 h period, this was not significant ($P=0.12$) (Table 5.34a hours 1 to 41). The proportion of time spent lying over the 41 h period was 70.4, 70.3 and 75.0% for cows in treatments C, 2 and 4, respectively. At the end of the 41 h period, when the deprivation period was included, treatments 2 and 4 were in deficit by 83 and 54 min, respectively, of their lying time, in comparison to treatment C, but this was not significant (Table 5.34a, hours – 4 to 41). Thus, over the course of the 41 h period, the treatment 4 cows gradually increased their lying time, in comparison to treatment C, to compensate for their lying deficit, whilst the treatment 2 cows lost their initial compensatory achievement thus not reducing their lying deficit. This loss of initial

compensation by the cows in treatment 2 appears to have occurred between the 17th and 24th hour, more specifically, during hours 10.25 and 12.25 (Fig. 5.4a), where they spent the most time standing and feeding, respectively, in comparison to the other two treatments.

Table 5.34a The mean time (min) spent lying every eight hours, for the first 40 hours immediately following lying deprivation, for the whole period (hours 1-40) and for the whole period including the deprivation period (hours-4-40), for all the experimental animals in the control (C), 2 h (2) and 4 h (4) treatments

Hour, post-deprivation. (actual time)	Treatment C	2	4	SED ±	P-value
1 – 8 (1425-2220)	209.5^{b}	231.8^{b}	274.0^{a}	10.37	**<0.01**
9-16[¶c] (2225-0620)	436.9 (8.3)	438.5 (8.4)	435.9 (8.3)	(0.50)	0.96
17-24 (0625-1420)	243.8	210.3	238.5	10.87	0.07
25-32 (1425-2220)	215.0	217.5	242.0	11.70	0.21
33-40[¶c] (2225-0625)	423.5 (7.6)	427.7 (7.8)	422.7 (7.6)	(0.54)	0.86
1-41 (1425-0625)	1528.5	1527.3	1614.0	33.6	0.12
-4-41 (1025-0625)	1668.3	1585.0	1614.0	34.81	0.24

[¶] Used back transformed data to calculate values. [c]Transformed values in parentheses are expressed as min^{-1}, number x 10^{7}. Bold P-values are used where P≤0.05. Values with different superscripts are significantly different (P≤0.05).

There was a significant negative correlation between lying and standing for cows in treatments C , 2 and 4 in the first eight hours following deprivation (r^2=81.6, P<0.01; r^2=65.9, P<0.01; r^2=85.4, P<0.01, respectively). Treatment 4 cows had a negative correlation between lying and feeding (r^2=19.4, P=0.05).

Cows in treatment C maintained a greater accumulated lying time than those in treatments 2 and 4 throughout the entire 41 h period (Fig. 5.5a). It took approximately 29 h for treatment 4 cows to achieve approximately the same lying time as cows in treatment 2 (950 versus 948 min, respectively). Neither treatment 2 or 4 cows achieved a similar lying time to cows in treatment C within the 41 h period. From Fig. 5.5a, it would appear that cows in treatments 2 and 4 made the greatest recovery time between the 7th and 15th hour, *i.e.* where the difference in accumulated lying time between the cows in these treatments and treatment C cows was the least. However, this looks to be only a short-term compensation measure, as this difference increases slightly after the 16th hour to the end of the observation period.

5.4.5.1a Latency to, and duration of, the first lying bout immediately following the first milking post-deprivation

Latency to the first lying bout decreased significantly, between treatments, with increasing deprivation time (Table 5.35a). There was an increase in the duration of the first lying bout, between treatments, with increasing deprivation time, but this was not significant at the 0.05 significance level.

Table 5.35a The average latency to (min), and the mean duration of (min) the first lying bout immediately following the first milking post-deprivation, for the experimental cows in the control (C), 2 h (2) and 4 h (4) treatments

Measure (min)	Treatment			SED ±	P-value
	C	2	4		
Latency*	25.0	20.0	12.5	-	**0.03**
Duration	63.0	68.5	81.0	8.74	0.12

*Median values.

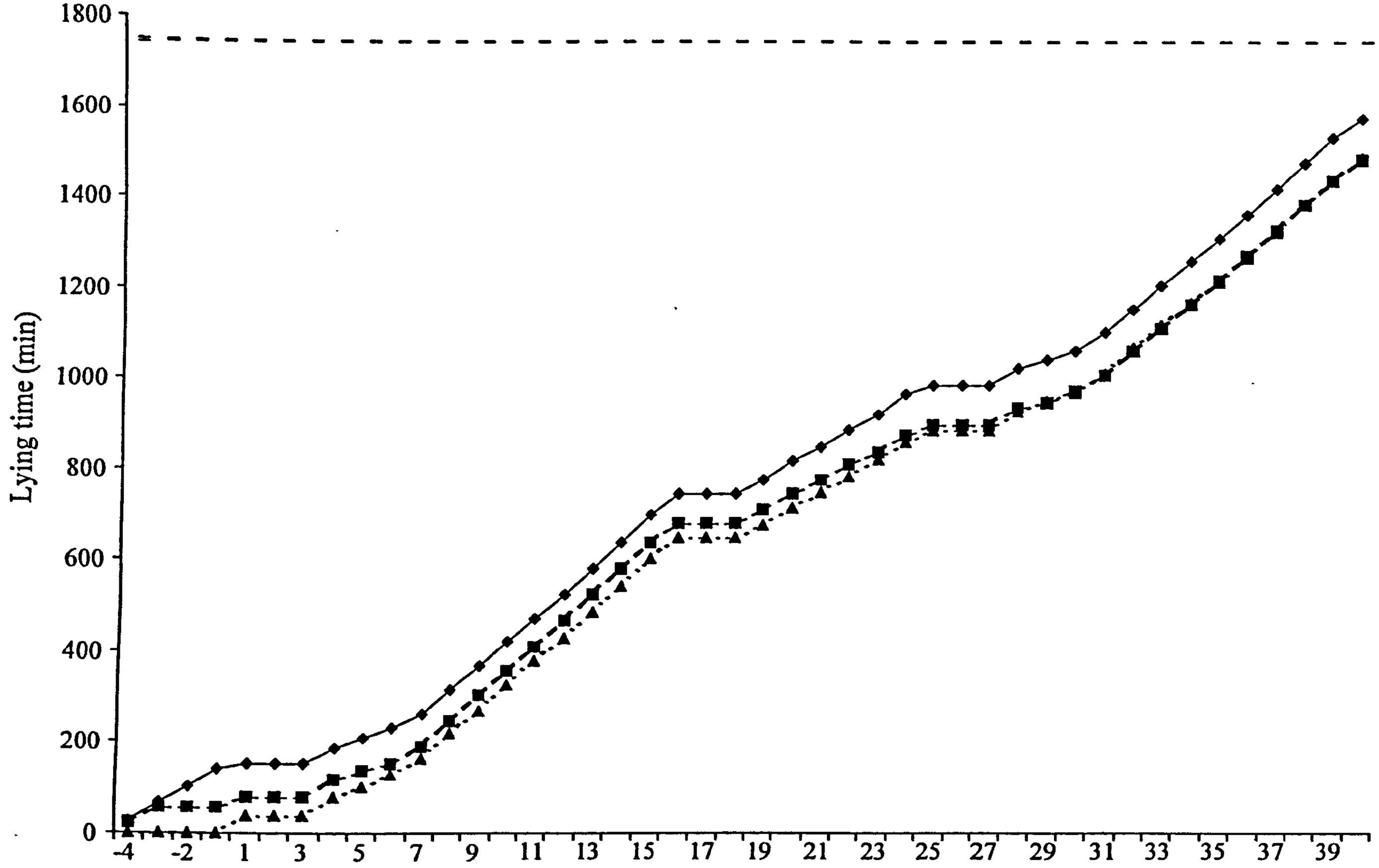


Fig 5.5a The accumulated lying time (min) per hour for all the experimental animals in the control ——◆—— , 2 h deprivation ---■--- , and 4 h deprivation ----▲---- treatments post deprivation (hours 1 to 40), and for the four hour deprivation period (hours –4 to –1) (I = SED bars)

5.5a Discussion

5.5.1a Milk yield recordings

The lower pre-experimental milk yield for the treatment 4 cows, although non-significant, is believed to be an unfortunate random allocation to treatment effect. This result appears to be primarily due to two treatment 4 cows (no. 30 & 73), which had particularly low milk yields in comparison to the other cows within their group.

Four hours of lying deprivation had a short-term impact on milk yield, as mean milk yields were significantly lower (aprox. 0.5 kg cow^{-1}) for cows deprived of lying for four hours compared to those deprived for up to two hours during the first milking post-deprivation, but not for the subsequent milkings. This reduction in milk yield is expected to be a real effect as the magnitude of this difference is greater than the pre-experimental difference. The initial reduction in milk yield is likely to be due to the stress of forced standing acting upon milk excretion. A similar effect has been observed in cows on their first day of oestrus. Horrell *et al.* (1984) and Schofield *et al.* (1991) observed that milk yield was negatively affected during the first milking post-oestrus, but not during subsequent milkings, due to alterations in milk let down rather than milk synthesis. The onset of oestrus is considered stressful due to increased activity between cows as they become more aggressive and compete with each other to elevate their position within the hierarchy and gain better access to the bull (Phillips, 1993).

Other studies have not shown milk yield to be significantly affected by lying deprivation (Munksgaard & Lovendahl, 1993; Verkerk *et al.*, 1999). However,

deprivation periods in these experiments lasted for several days and milk yields were either taken towards the end of the experiment or taken daily, but averaged for the duration of the experiment for analysis. Both methods may have enabled the cows to adapt to the stress over time, and also for short-term changes in milk yields to go undetected.

5.5.2a Continuous recordings

Leg raising, repositioning and weight shifting behaviours all increased in frequency with time spent in forced standing. In addition, head swinging and butting, although rarely observed, also increased in frequency in the deprived cows during the deprivation period. Weight shifting in response to lying deprivation in dairy cows has also been observed in other studies (Ruckebusch, 1974; Hopster *et al.*, 2002). The combined behaviours, shifting, restlessness-, stress- and frustration-related, showed the same pattern of increase, presumably because they were formed from combinations of the individual behaviours above. Behaviours such as leg raising, repositioning, and weight shifting may enable the cow to cope by alleviating strain on the legs and hoofs. Regular movement, or shifting, may temporarily prevent or reduce discomfort. Head swinging and butting, on the other hand, are more likely to be behavioural expressions of subjective feelings. These behaviours have previously been shown to be responses to frustrating and stressful situations in dairy cows (Sandem *et al.*, 2002). Other researchers have also suggested that aggression, such as butting, can be performed as a result of frustration (Dollard *et al.*, 1939; Miller *et al.*, 1941; Scott, 1948). Head swinging has also been observed in other animals experiencing frustrating situations (Cook, 1992). Frustration, in this case, could have arisen as a result of not being able to lie down, only being able to lie on a relatively

uncomfortable surface, and/or due to the negative impact of forced standing. In addition to these effects, the increased butting activity may have partly been due to being confined during deprivation, as animals have been shown to be more aggressive when space allowance is reduced (Grigor *et al.*, 1997; Grasso *et al.*, 1999). As the frequency of these behaviours increased with deprivation time, this may suggest that the negative physical and psychological effects of lying prevention, and the motivation to lie, are cumulative. This would imply that the longer the deprivation period, the more severely an animal's welfare is compromised. It is also possible that the increased frequency of these behaviours, to some extent, could be attributed to boredom, as animals housed in barren environments have been shown be more active (Stimulus Response, 1995).

The tendency for ground sniffing behaviour to decline, between treatments, with increasing deprivation time is most likely to be related to the absence of lying, as the experimenter observed this behaviour taking place prior to lying in the treatment C cows. It appeared that this activity was performed in order to select, or assess, a suitable lying area. As the deprived cows were prevented from lying, the requirement to ground sniff was therefore reduced. The deprived cows still executed this behaviour maybe because they wanted to, or considered, lying.

Over the entire deprivation period, the treatment 4 cows drank more frequently and for longer and tended to lick more frequently than the other treatments. This finding is in agreement with other studies, whereby cattle deprived of lying groomed themselves more often and increased the frequency of oral behaviours, *i.e.* licking or chewing stall fixtures (Munksgaard & Simonsen, 1996; Munksgaard *et al.*, 1999). Ruckebusch

(1974) also observed stereotypical oral activities in cows thwarted of lying for periods of up to 22 h per day. It is plausible that these behaviours were performed to compensate for a lack of stimulation within the deprivation environment (Kerr & Wood-Gush, 1987; Stimulus Response, 1995; Munksgaard & Simonsen, 1996; Hansen & Berthelsen, 2000), rather than as a direct result of the stress of lying prevention. These behaviours may be less likely to be observed if cows were not confined, or were deprived of lying whilst at pasture. The increased frequency and duration of drinking could also be in response to the stress experienced due to forced standing by the treatment 4 cows. Polydipsia has been shown to occur during periods of stress in other animals (Tarjan & Denton, 1991; Rodriguez de Turco *et al.*, 1993; Howell *et al.*, 1998).

5.5.3a Behavioural versus production variable correlations for continuous recordings

It is expected that the increasing frequency of leg raising, weight shifting, shifting, restlessness- and stress-related behaviours with increasing number of days in milk, for the treatment 4 cows, was due to their stage of pregnancy. The treatment 4 cows were approximately five months pregnant. The pressure of the foetus may have caused some discomfort whilst standing, and possibly also put extra strain on the limbs, which may have been eased by regularly shifting body weight between the limbs and moving the legs. The treatment 4 cows tended to increase restless-type behaviours as the number of days to parturition increased, and decrease leg raising with increasing milk yield, which may also support this view. A similar reasoning may also be used to explain why the treatment C cows increased restless-related behaviours and had a tendency to increase leg raising and shifting behaviours in response to increasing milk

yields. These behaviours may have been employed to reduce possible strain on the legs caused by an additional milk load.

A time availability effect is likely to be responsible for the increasing frequency of self grooming, rubbing head against housing, body care and licking with increasing days in milk, for the treatment C cows. With the exception of rubbing head against the housing, these behaviours can be regarded as orally-centred body care activities. It is expected that those cows towards the end of their lactation have more time to perform these behaviours due to spending less time in other activities. The treatment C cows became less active, *i.e.* walked and interacted with the housing less frequently, as the number of days to parturition increased, and became more restless and tended to shift and leg raise more often as milk yield increased. This supports the above view because the number of days in milk is linked to both the number of days to parturition and milk yield. It is also possible that these cows, which are not occupied with other activities, and housed within a barren environment, engage in such behaviours as a form of stimulation (Kerr & Wood-Gush, 1987; Stimulus Response, 1995). The increased time spent drinking as the number of days in milk increased, and the positive relationship between age and head swinging for these cows, may have also been performed for the same reasons. Significant correlations between these variables were not observed in treatments 2 or 4, possibly because they were performing these behaviours anyway, due to the effect of lying deprivation, and therefore regardless of lactation stage. Similarly, this may also be the reason for leg raising not being significantly correlated with body condition score for treatments 2 or 4.

The thinner cows in treatment C leg raised more often than the fatter cows. Wildman *et al.* (1982) reported an inverse relationship between milk yield and body condition score in dairy cows, and Veerkamp *et al.* (1994) observed that higher yielders have a significantly lower mean body condition score in comparison to lower yielding cows. This relationship was tested and there was a negative correlation between milk yield and body condition score for these cows (r^2=28.6, P=0.02). Thus, the thinner cows were higher yielding and leg raising behaviour may have been performed to reduce discomfort caused by the extra stress on the legs due to an increased volume of milk within the udder.

As sniffing may be linked to the preparatory phase proceeding lying, this may explain why the older treatment 2 cows spent more time sniffing the housing, as these cows spent longer lying ruminating.

Repositioning in treatment 2 cows and walking in treatment C cows decreased with the progress of pregnancy. This may be explained as cows tend to become less active, for example, by avoiding aggressive interactions and social encounters, in the later stages of pregnancy, possibly as a cautionary measure to safeguard the foetus (Phillips, 1993). Veris *et al.* (1980) also observed that cows become less active, by increasing the time they spend lying, as the number of days to parturition increases. Lying may help to reduce discomfort caused by the pressure of the foetus and also reduce time spent supporting the extra weight.

5.5.4a Behavioural correlations for continuous recordings

The frequency and time spent drinking both increased with increasing self grooming, body care and licking behaviours for all treatments, which may demonstrate the additional requirement for water, possibly to replace that lost as saliva, whilst performing these behaviours. Increased drinking may also be used to help lubricate the tongue, or rinse the tongue, after grooming. It is also possible that these behaviours increased together as they may be performed in response to a lack of stimulation within the environment, which may explain why the frequency and duration of rubbing the head against the housing increased with drinking frequency and duration for all treatments. This may be supported further as drinking time was positively correlated with leg raising, (treatment 2), stress-related behaviours (treatment 4) and weight shifting (treatment 4), which are presumed to be expressions of frustration. Alternatively, these latter correlations could be interpreted as stress-related polydipsia (Tarjan & Denton, 1991; Rodriguez de Turco *et al.*, 1993; Howell *et al.*, 1998), as they were only observed in the deprivation treatments. Although possible, this may be refuted as self grooming, the number of licks whilst self grooming, licking and body care behaviours all decreased with increasing frustration-related behaviours, in the treatment C cows. However, the frustration experienced by the deprived cows may be different, and have arisen from a different source, to that experienced by the control cows. The treatment C cows may have been frustrated due to a lack of stimulus within the environment, whilst the deprived cows' frustration may have arisen primarily from forced standing. Different experiences, or sources, of frustration may give rise to alternative behavioural displays. Another explanation is that as the lying deprived cows spent more time drinking, they would have also been standing only for longer and thus the incidence of leg raising, stress type behaviours

and weight shifting would also be greater in these cows. This reasoning may be especially suited to weight shifting behaviour, as this was also observed in the treatment C cows. Orally centred and body care type behaviours may also be pleasurable activities, rather than coping behaviours, and performed in response to a positive, or the absence of a negative, mental state, as the treatment C cows executed these behaviours more frequently as the performance of frustration-related behaviours declined.

Sniffing and ground sniffing were positively correlated with frustration- and stress-related behaviours, for the treatment 4 cows. The treatment 4 cows may have wanted to lie and so sniffed the ground, but being deprived of doing so, may have caused them to become frustrated and stressed. These behaviours, for these cows, were also positively correlated with restlessness-type behaviours, shifting, weight shifting and stress-type behaviours (treatment 2 also), which are regarded as possible indicators of frustration and stress, and thus may further support a relationship. Sniffing housing also increased with increasing restlessness-related behaviours, for treatment 4 cows, which may provide further evidence that sniffing behaviours, in this case, are indicative of frustration. Frustration-, restlessness- and stress-related behaviours all increased with housing interaction, in treatment 4 cows, which is probably due to it being a function of sniffing the housing. Conversely, for cows in treatment C, the less frustration-related behaviours they displayed, the more they interacted with the housing, possibly because they chose to lie down when they were less frustrated.

Self grooming (treatments C and 2), and the number of licks whilst self grooming (treatments C and 4), were positively correlated with housing interaction, possibly

because housing interaction is a function of rubbing of the head against the housing, which can also be considered as a grooming or body care activity. Rubbing the head against the housing was positively correlated with self grooming (treatment 4), the number of licks whilst self grooming (treatments 2 and 4) and licking behaviours (all treatments). Similarly, the time spent rubbing the head against the housing increased with the number of licks whilst self grooming (treatments 2 and 4), and the frequency of self grooming (treatment 2). This demonstrates that these behaviours form a set of similar actions that respond in a similar way in different cows.

It is assumed that butting and head swinging for the cows in treatment 2 increased together as the origin of their performance is similar, *i.e.* they are both potential indicators of frustration.

It is likely that head swinging was negatively correlated with weight shifting and shifting, for the cows in either treatment C or 2, as weight shifting and shifting were enough to ease any discomfort and therefore reduce frustration, and thus the performance of head swinging. Leg raising and head swinging may be expected to increase together in the treatment 4 cows, as they are both behaviours assumed to be related to the discomfort and frustration of lying deprivation. This may also explain the positive correlation between repositioning and weight shifting in the treatment 2 cows.

It is likely that in the early stages of deprivation, leg raising activity was enough to temporarily relieve any possible discomfort the animal was experiencing. As the length of time deprived of lying increases, this behaviour may be replaced by more

active responses, such as repositioning, which may explain the negative correlation between these behaviours in the treatment 4 cows.

The cows in treatment C were observed increasing licking behaviours the more frequently they sniffed the ground. They may have increased together as they can both be regarded as oral exploratory behaviours. An alternative explanation is that ground sniffing was observed taking place prior to lying, and grooming behaviour, a contributor to licking behaviours, has been shown to occur whilst animals are in a restful state (Lam, Rupniak & Iversen, 1991; Eckstein & Hart, 2000) and is induced by oxytocin (Van Erp, Kruk & De kloet, 1993). This is supported, as grooming self also increased with ground sniffing and sniffing the housing, and the number of licks whilst grooming self was positively correlated with sniffing housing for these cows. Likewise, self grooming and the number of licks whilst self grooming were positively correlated with sniffing behaviours, for the cows in treatment C. There was also a positive relationship between grooming self and ground sniffing for both deprivation treatments. This may be because increased self grooming can also be an indicator of stress (Van Erp *et al.*, 1994; Munksgaard & Simonsen, 1996; Hansen & Berthelsen, 2000; D'Aquila *et al.*, 2000). It is important to mention that it may not just be the frequency of grooming that is possibly indicative of stress, but also the timing of this activity. For example, grooming prior to, or during rest, may be considered as normal, whereas grooming at other times, such as when encountering a particularly frustrating situation, could be indicative of stress. For example, Munksgaard and Simonsen (1996) demonstrated that cows increase grooming activity when exposed to a stressful situation, indicating frustration. In addition, rats have been shown to excessively groom in response to the stress hormone adrenocorticotrophin (Van Erp, Kruk & De

kloet, 1993). The deprived cows possibly sniffed the ground as they wanted to lie, but the thwarting of this behaviour induced stress and frustration, which manifested itself as grooming behaviour. This may be supported by the observation that licking behaviours increased with weight shifting for treatment C cows, also. The experimenter observed some cows standing for long periods prior to lying, which may have been when weight shifting occurred in these cows. Furthermore, treatment C cows were observed weight shifting more frequently as sniffing the housing, the time spent sniffing the housing, ground sniffing and sniffing behaviours increased, which were observed taking place pre-lying. Conversely, treatment 2 cows were also observed licking more frequently the more they shifted their weight. This contradiction is likely to be explained in the same way as that of grooming self and ground sniffing above. Body care activities, which includes grooming behaviour, also increased with weight shifting, for all treatments.

Leg raising, restlessness type behaviours and shifting all increased the more frequently treatment 2 cows were observed walking. It is likely that the former behaviours substituted for walking during the deprivation period. Maybe those cows observed walking more frequently were more affected by the limited space allowance and thus more readily redirected this behaviour to leg raising, restlessness type behaviours and shifting. It is expected that some discomfort came from the limited area in which the cows could move, as opposed to just being deprived of lying.

5.5.5a Instantaneous recordings

The deprived cows appeared to primarily replace lying time with standing and feeding. They spent longer feeding in their first hour of deprivation, which may have

provided them with an activity to perform in a situation where there was little else to do, *i.e.* compensated for the increased time spent standing. Increased feeding has also been observed where horses were prevented from crib-biting (McGreevy & Nicol, 1998), which may further demonstrate that this behaviour has a compensatory effect. Ingvartsen *et al.* (1999) did not observe greater feed intakes in lying deprived cows. This may have been because their cows were subject to 14 h of lying deprivation daily for 10 weeks, thus any initial increase in feed intake, possibly within the first few hours of deprivation, went undetected. It is unlikely that the deprived cows could continually increase their feed intake over a 14 h period and maintain this for 10 weeks. However, Munksgaard and Simonsen (1996) did observe cows that were deprived of lying for 14 h daily for eight weeks to have a greater frequency of eating, in comparison to the control cows.

Ruminating predominantly took place whilst lying, as standing ruminating was rarely observed for the treatment C cows over the entire deprivation period and for the treatment 2 cows in the first two hours. This is supported by Phillips (1993), who stated that rumination often takes place during periods when cows are less alert, thus is likely to be associated with periods of rest. Additionally, standing ruminating only typically takes place for approximately 0.04% of the day in comparison to 22.9% for lying ruminating, in lactating dairy cows (Phillips, 1993). The increase in time spent standing ruminating, between treatments, with increasing deprivation time would therefore be expected to be related to the deprivation of lying. These results are in agreement with those of Munksgaard and Simonsen (1996) and Munksgaard *et al.* (1999), who noted that lying deprived cows spent a greater proportion of their time ruminating standing in comparison to control cows. As lying ruminating is performed

more than standing ruminating, this may explain the reduced time spent ruminating in the first two hours of deprivation by the cows in treatment 4 – because they were unable to lie they were less inclined to ruminate. Possibly, standing ruminating increased between hours over the deprivation period for the cows in treatment 4, as the need to ruminate accumulated, due to the earlier suppression of this behaviour and/or due to the increased time spent feeding. To spend more time ruminating it appears that the cows decreased the amount of time spent standing only, as this was the only behaviour to gradually decline during this period. This may explain why the treatment 2 cows spent nearly twice as much time only standing during actual deprivation, in comparison to the previous two hours, as they had not fed for as long as the treatment 4 cows, and had spent time ruminating prior to deprivation, thus the requirement to ruminate may not have been as great. It appears that ruminating behaviour was inhibited by lying deprivation, as the proportion of time spent ruminating (both lying and standing) decreased, between treatments, with increasing deprivation time (38, 28 and 23%, for treatments C, 2 and 4, respectively). Phillips (1993) stated that ruminating can be used as an indicator of contentment and that its absence can be associated with stress.

The treatment C cows were observed lying for approximately equal amounts of time in each hour of the deprivation period, for a total of 54 min. The cows in treatment 2 were also observed lying for approximately equal amounts of time in the first two hours, for a total of 26 min. This indicates that the cows were motivated to lie throughout the entire deprivation period. Although the treatment 4 cows were deprived of lying for four hours, during the deprivation period the treatment C cows only spent a mean time of 140 min (2 h 19 min) in lying behaviours (lying & lying

ruminating). Thus, the treatment 4 cows were, in real terms, only deprived of lying for this amount of time. Likewise, the treatment 2 cows were only deprived of lying for 82 min.

5.5.6a Behavioural versus production variable and behavioural correlations for instantaneous recordings

The decrease in feeding time with increasing milk yield, for the treatment 2 cows, is contrary to previous findings (Chapters 3b and 4). Other studies have shown these variables to be positively correlated (Lathrop *et al.*, 1988; Phillips & Denne, 1988; Bao *et al.*, 1992; Gibb *et al.*, 1999), indicating increased feed intake with increasing milk yield. It is possible that the higher yielding cows started feeding before the lower yielding cows, but this went undetected, as behavioural observations were not taken prior to the deprivation period. It is also possible that the higher yielding cows may have been eating faster, and thus intake per unit time was greater. This has been observed in high yielding grazing dairy cows (Chapter 3b) (Gibb *et al.*, 1999; O'Connell *et al.*, 2000).

The cows in treatment 4 spent less time standing as milk yield increased, possibly due to the corresponding increase in standing ruminating – due to the additional time spent feeding throughout the deprivation period. The reduced time spent in all standing behaviours as milk yield increased, in the deprived treatments, may also be due to the additional time spent ruminating and feeding.

Feed intake declines as pregnancy advances, and rumen capacity declines as a result of the presence of the foetus (Phillips, 1993), thus cows further into pregnancy may

ruminate more frequently as gut fill is achieved more quickly, which may account for the positive relationship between standing ruminating and the number of days to parturition, for the treatment 2 cows.

As milk yield increases from lactation one to eight (Phillips, 2001), it may be that higher yielding cows have a greater feed intake and thus spend longer periods ruminating, which may account for the positive relationship between lactation number and lying ruminating for the treatment 2 cows. The tendency for the treatment 2 and 4 cows to increase standing time with lactation number could be due to standing, rather than standing ruminating, replacing lying ruminating during deprivation. This may be further supported, as there was a negative correlation between ruminating lying and feeding for the treatment 2 cows.

The time spent feeding and standing were negatively associated for the treatment C cows, indicating a trade-off between these activities, presumably because these cows were not prepared to reduce their lying time to increase their time spent feeding. The negative relationship between these behaviours for the treatment 4 cows was expected, as there was little else they could give up in order to feed, except standing ruminating.

5.5.7a Lying positions and changes in posture post-deprivation

It is possible that cows determine a probable comfortable lying position prior to lying, as changes in posture were not observed in any of the treatments once the cows had lied down. Alternatively, changing posture may be difficult to perform once lying and, unless particularly uncomfortable, the cow may settle in the adopted position. It

is assumed that the lateral lying posture was preferred over the sternal lying posture, as only one cow was observed in the latter.

5.5.8a Post-deprivation video observations

The cows in treatments 2 and 4 significantly reduced their feeding time in the first eight hours post-deprivation, in comparison to the treatment C cows. This reduced time spent feeding by the treatment 4 cows approximately matched that of the additional time spent feeding during deprivation (35 versus 30 min, respectively). The cows in treatment 2 fed for 26 min less than the treatment C cows in the first eight hour period, which was twice as much as their increased feeding time during deprivation. This deficit did not motivate the cows in treatment 2 to significantly increase their feeding time, in comparison to treatment C cows, in the subsequent eight hour periods. However, there was a compensatory increase in feeding time for the treatment 4 cows in the subsequent eight hour period, compared with the treatment C cows, but the degree of difference in feeding time between these treatments was considerably less than that within the first eight hour period (+ 12 versus - 35 min, respectively). Thus, within the first 16 h post-deprivation, the treatment 4 cows reduced their overall feeding time by 22 min, compared to the treatment C cows. Over the 41 h period, the treatment 4 cows spent a total of 54 min less time feeding than the treatment C cows, but this difference was not significant when the feeding time during the deprivation period was included. The results suggests that for both deprivation treatments it was the increased time spent feeding during the deprivation period that facilitated the reduction observed during the first eight hour period. This re-scheduling of feeding behaviour probably prevented feed intake being compromised long-term. This is a useful strategy, but has not been observed where dairy cows were

deprived of lying for longer periods (Ingvartsen *et al.*, 1999; Munksgaard & Simonsen, 1996), suggesting that it is not possible to achieve over a longer period of deprivation. There is strong evidence to suggest that it was this reduction in feeding time that enabled the cows to increase their lying time, as the greatest differences in feeding times between the deprived treatments and treatment C, coincide with the greatest differences in lying times between these treatments (hours 1, 2, 5 & 6, post-deprivation). It was also necessary for the treatment 4 cows to reduce standing time in order to increase their lying time, which indicates that adjusting feeding time alone was not sufficient to ensure the maintenance of this behaviour.

The prevention of lying for treatments 2 and 4 resulted in the greatest increase in lying time, in comparison to treatment C, during the first eight hour period in comparison to any other eight hour period post-deprivation. Treatments 2 and 4 compensated for about 27% (22 min) and 46% (65 min), respectively, of their actual deprived lying times within this period. Similarly, Metz and Wierenga (1984) revealed that cows deprived of lying for three hours compensated for 53% of their total lying deficit in the first seven hours post-deprivation and Metz (1985) found that cows recovered 58% of their three hour deprived lying time within 10 h. Fisher *et al.* (2003) also observed cows increasing their lying times whilst at pasture for a three hour period immediately after nearly five hours of lying deprivation. This initial rebound in lying time for the treatment 4 cows, in comparison to the treatment C cows, indicates that the need for lying builds up significantly after only a few hours of lying deprivation. Several researchers have come to a similar conclusion (Metz & Wierenga, 1984; Metz, 1985; Munksgaard & Simonsen, 1996). This is demonstrated further as the motivation to lie in the first hour after milking post-deprivation

increased proportionately with increasing time spent deprived of lying, as demonstrated by the latency to the first lying bout. The duration of the first lying bout also increased between treatments with increasing deprivation time but was not significant. This is in line with results of Hopster *et al.* (2002), who observed longer lying bouts in dairy cows deprived of lying for six but not three hours. Within the 41 h period, the additional lying time of the treatment 4 cows, in comparison to the treatment C cows, matched that of their deprived lying time (86 versus 87.5 min, respectively), indicating they were able to compensate for their reduced lying time within the 41 h period. Correspondingly, Hopster *et al.* (2002) observed that three hours of lying deprivation had no effect on lying times of dairy cows over a 24 h period, but cows deprived of six hours lost 106 min of lying time, in comparison to the control cows.

The negative relationship between standing and lying for all treatments in the first eight hours post-deprivation suggests that they would reduce time spent standing rather than feeding, to increase lying time. However, for the cows in treatment 4, lying was also negatively correlated with feeding during this time, indicating a trade-off between these activities whereby one would be pursued at the expense of the other.

It took 29 h for the cows in treatment 4 to achieve approximately the same lying time as the treatment 2 cows. However, neither cows in treatments 2 or 4 achieved 100% lying compensation within 41 h when the four hour deprivation period was also taken into account, *i.e.* their accumulated lying time did not match that of the treatment C cows. This may suggest that two hours of lying deprivation was not severe enough to

motivate the cows to decrease their lying deficit, as it would have been expected that the treatment 2 cows would have achieved the same lying time as those in treatment C in approximately 29 h. Other studies concerned with the deprivation of lying in dairy cows have also observed incomplete compensation (Metz, 1985; Munksgaard & Simonsen, 1996; Munksgaard *et al.*, 1999). Metz (1985) suggested that a 100% recovery rate is unlikely to be found as cows deprived of sleeping never make a complete recovery. However, it could be that the decreased time spent feeding by the cows in treatment 2, in comparison to the treatment 4 cows, during the deprivation period, and the additional reduction in feeding time in the first eight hours post-deprivation, in comparison to the treatment C cows, made it difficult to give up additional feeding time in which to lie. This may have been why the treatment 2 cows spent longer feeding in the 17^{th} and 24^{th} hours post-deprivation, compared to the cows in the other treatments, and as a result lost their initial compensation in lying time achieved during the first eight hour period. Full compensation may also only be achieved over a longer period. Dairy cows deprived of lying for six hours per day for one week, took three days to completely compensate for their daily deprivation (Hopster *et al.*, 2002).

In conclusion, prevention of lying, even for two hours, poses a risk to cow comfort. Behavioural observations during deprivation demonstrated signs of fatigue, frustration *and stress. The negative* physical and psychological effects of forced standing were cumulative, indicating a persistently increasing impact on welfare with time. Four hours of lying deprivation resulted in an immediate rebound effect post-deprivation, demonstrating that lying can be regarded as an important and highly motivated activity, and its prevention can compromise welfare. It appears that a re-scheduling of

feeding behaviour allowed lying time to be increased with little long-term change in the time devoted to this behaviour. Four hours of lying deprivation also reduced milk yields by approximately 0.5 kg cow^{-1}, further illustrating the requirement to perform this activity. The findings of this study would suggest that preventing cows of lying, even for a short period, should be avoided.

The actual lying deprivation time undergone by the cows in treatment 2 mimics that experienced by the grazing high yielding dairy cows in a previous experiment (Chapter 3b) (82 versus 61 min, respectively), thus adding value to the validity of the transference of these results to such cows. However, there are potential confounding factors, which could make a direct comparison implausible. For example, the cows were deprived of lying in one bout. As lying is a polyphasic activity, it is more likely that high yielding cows have a number of shorter lying periods in comparison to their lower yielding counterparts. The experiment also only examined the effects of lying deprivation for one day. It is likely that grazing high yielding dairy cows are deprived of lying every day, possibly for the duration of their lactation. This may lead to cumulative effects, which could not be observed in this study. A further dissimilarity is that the deprivation of lying took place within a small area, which restricted movement. This would not be a situation experienced by grazing dairy cows. It is probable that some of the behaviours expressed, such as leg raising and weight shifting, were symptomatic of standing for long periods in a confined space, rather than being deprived of lying *per se*. The immediacy in which to lie after deprivation may have also been largely affected by this factor. The results of this study, in some instances, may therefore be relevant to the effects of long periods of standing, for example, whilst awaiting veterinary attention or artificial insemination, when

confined to concrete yards during periods of wet weather, or due to over-occupation of cubicle housing, on dairy cattle welfare.

There is also a problem with the construal of behaviours. It is difficult to interpret exactly what a rebound result means in terms of welfare to the animal, *i.e.* how much of a rebound constitutes suffering (Dawkins, 1988). It lacks a yardstick for truly comparative purposes. It would be useful to use, for example, food deprivation as a comparison. Food is a useful comparator as it is known to be an essential resource and its deprivation results in suffering (Dawkins, 1988). Maybe depriving cows of both food and rest simultaneously would also better reflect the situation faced by the high yielding cow, as the grazing high yielder is both motivated to perform both of these activities at the same time.

5b. The statistical analysis of paired dairy cows I

5.1a Introduction

Concern has been expressed regarding the validity of using individual dairy cows as replicates in the statistical analysis of their behaviour (Rook & Huckle, 1995). As cows are social animals (Phillips, 1993) the possibility exists that the behaviour of individuals could be influenced by other cows. For example, Rook and Huckle (1995) believe that the grazing behaviour of dairy cows may be socially facilitated, with the commencement and duration of this period being dependent on a few individuals within the group. This assumption does not extend to all behaviours. There are, of course, some behaviours, such as allogrooming, aggression and yawning, which must be interdependent. Conversely, respiration, for example, is almost certainly independent. Other behaviours, primarily feeding, standing and lying, are not so easily categorised and are the subject of controversy. If such behaviours were shown to be interdependent, then this could nullify the use of certain statistical techniques, such as analysis of variance (ANOVA), for data exploration. The use of ANOVA assumes that individuals are behaving independently (Snedecor & Cochran, 1978), a contravention of this assumption would invalidate the use of this test as a means of statistical analysis. Currently, researchers must ensure that data collected from animals within groups is handled in such a way as to cater for these possible allelomimetic effects. Typically, one of two methods can be employed to manipulate the data, thus making it suitable for ANOVA tests. The experimenter can either use the data of one animal from each replicate for statistical analysis, *i.e.* have a 'focal cow', or combine the data for all the cows within a replicate and use the mean value. Both methods will provide data conforming to the assumptions of an ANOVA test and therefore justify its use. However, although such manipulations achieve the status

of independent units, there are problems with the resulting data. First, the data being used for analysis will still be the product of data that has been influenced by allelomimetic effects and would be expected to be different from that given if animals were behaving independently. For example, suppose the time spent feeding was recorded for two cows (cow 'a' and cow 'b'), which were housed separately (Fig. 5.1b – scenario one). As there is no interaction between the cows they will be behaving independently of each other. Under these conditions their feeding times may differ and cow 'a', for example, may have a greater feeding time than cow 'b'.

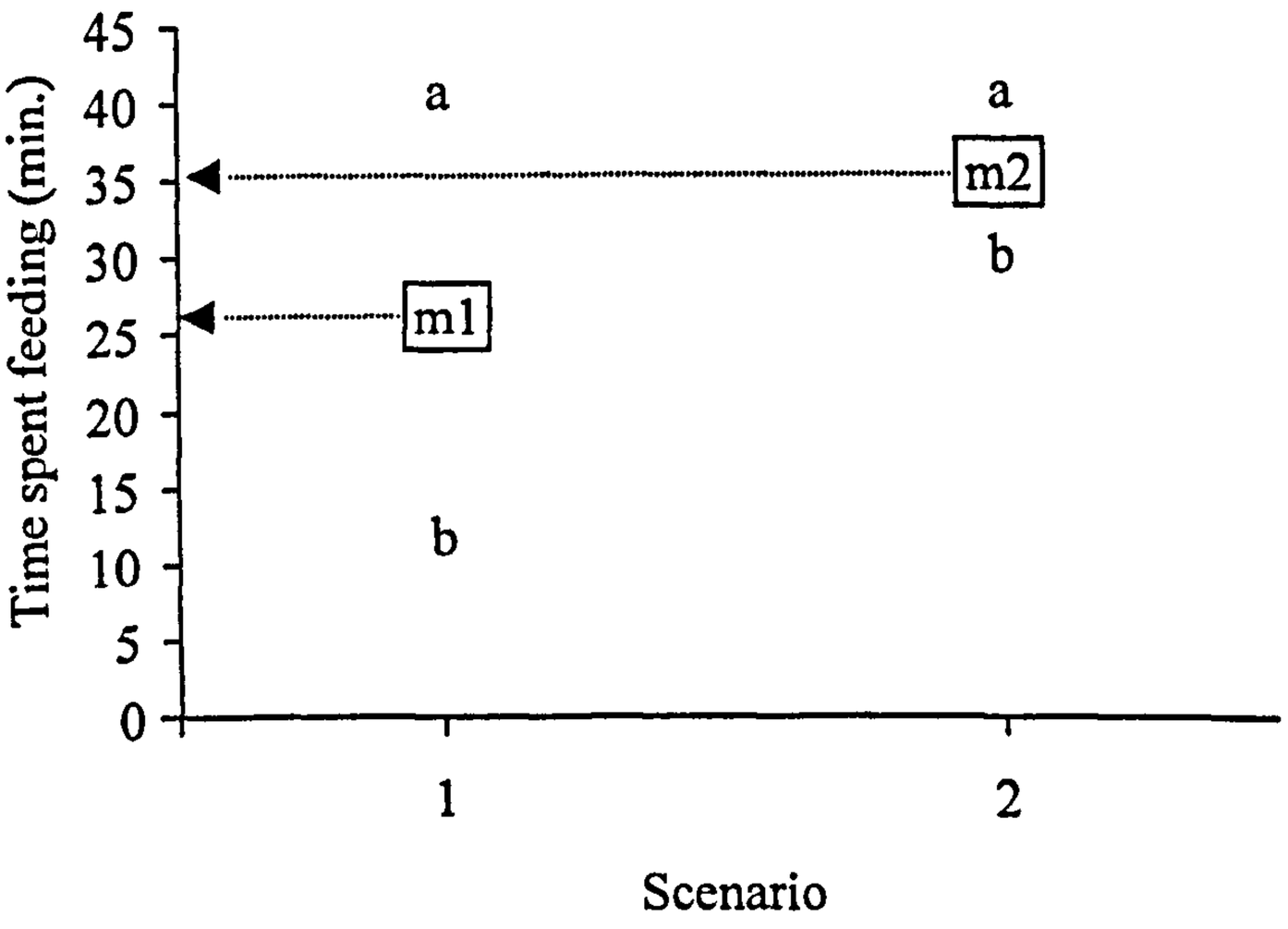


Fig. 5.1b Feeding times of two cows ('a' and 'b') when housed separately (scenario 1) and together (scenario 2). Mean feeding time for cows in each scenario is represented by 'm'.

If the two cows were then housed together, and the occurrence of interdependence is accepted, they would behave similarly, as one of the cows will be dictating the behaviour of the other, for example, cow 'a' may dictate the behaviour of 'b', therefore increasing its feeding time (Fig. 5.1b – scenario two). When it comes to analysing the data, cow 'a' could be taken as the 'focal cow'. However, this value, *i.e.*

its own feeding time, is only representative of itself in scenario one, but considerably overestimates that of the other cow. Conversely, if cow 'b' was used as the 'focal cow', then this value both underestimates that of cow 'a' and also overestimates that of itself. If the alternative procedure was followed, *i.e.* the mean feeding time was taken for the two cows (m2), then this value is also very different from that which would be calculated if they were behaving independently (m1). This means that whichever method is used to obtain the value used for statistical investigation, both methods provide values which are different to those obtained from independently behaving cows.

Secondly, in both cases, data is being 'lost': The amount of data available for analysis is reduced. This ultimately increases the standard error due to a reduction in the amount of variation within the data set, and therefore makes a type II error more likely, *i.e.* a significant difference less likely to be revealed.

Allegations of interdependence have primarily arisen from an observation of the synchronous behaviour of dairy cows whilst grazing (Rook & Huckle, 1995). However, the existence of further evidence to support this is absent. The aim of this study, therefore, was to determine whether dairy cows, whilst performing behaviours such as feeding, standing, and lying, can be considered to be acting independently. This can be achieved by comparing the variation between individuals within a group with the variation between individuals from different groups, for a number of behaviours. Variation is a useful measure as it provides a measure of the degree of dissimilarity of the expression of a behaviour between individuals. Cows with similar patterns of behaviour could be considered as behaving interdependently, and the

variation between these individuals would thus be relatively small. On the other hand, a greater degree of variation between individuals would indicate that they are behaving differently, is suggestive of behavioural independence and would not be so likely if cows within the same group were influencing each other's behaviour.

The data used for analysis was from 40 cows, which were housed in pairs. Hence, there were individuals that could interact with each other, *i.e.* cows within the same pair, and individuals that were unable to influence each other's behaviour, *i.e.* cows not within the same pair. Individuals within the same pair were used to calculate the degree of variation present when cows were able to influence each other's behaviour. The variation existing between individuals unable to influence each other's behaviour was calculated by comparing the degree of variation present between individuals not within the same pair. Therefore, within-pair and between-pair variation was compared. If there is no evidence for a difference between the variables examined, this could imply independence of behaviour within groups. If it can thus be shown that dairy cows behave autonomously, then this would be evidence to legitimise the use of individuals within groups for statistical analysis, rendering current cautionary measures and data loss through data manipulation unnecessary.

5.2b Materials and Methods

5.2.1b Animals, conditions and experimental conditions

The animals, conditions and experimental conditions are as those in Chapter 5a, section 5.2a.

The behaviours used for analysis were collected instantaneously during the deprivation period, except for leg raising behaviour, which was collected continuously over the same period (see Chapter 5a, section 5.2.6a).

5.3b Statistical analysis

All statistical analyses were performed using the 13th release of the Minitab statistical programme (Minitab, 2000).

For all of the behaviours except leg raising, only cows in treatments C and 2 were used for analysis. Cows in treatment 4 were not used as by the nature of their treatment they were unable to perform certain behaviours, *i.e.* lying and ruminating lying. For leg raising behaviour, cows in treatment 4 were used instead of those in treatment C, as cows in the latter treatment only occasionally performed this behaviour, thus insufficient data was available for any useful statistical analysis.

Two methods of analysis were employed. The first method used the data collected from all four hours of the deprivation period, *i.e.* the total time (min) spent in each behaviour during this period. The six mutually exclusive behaviours used for analysis were; feeding, standing, lying, ruminating whilst standing, ruminating whilst lying and leg raising. To determine the variation between cows within the same pair, *i.e.* cows that were interacting and able to influence each other's behaviour, each cow within each pair was randomly assigned to one of two groups for analysis. Hereafter this will be termed the 'within-pair' analysis. This gave rise to 20 cows per group. To determine the degree of variation between cows not within the same pair, *i.e.* cows that could not interact and were unable to influence each other's behaviour, each pair

of cows was randomly assigned to one of two groups for analysis. This gave rise to ten pairs of cows per group. The random designation of pairs to groups was conducted to avoid any treatment effects. Hereafter this will be termed the 'between-pair' analysis.

The second method only used the total time (min) spent in each behaviour during the first two hours of the deprivation period. During this period the treatment 2 cows were not being deprived of lying and were therefore under the same conditions as the treatment C cows, and for the same time. Four mutually exclusive behaviours were used for analysis: Feeding, standing, lying, and ruminating whilst lying. The data collected for ruminating whilst standing during this period was not used due to a high number of zero values, therefore it was not normally distributed and was resistant to any normalisation procedure. Insufficient data was collected for leg raising behaviour during this period for any useful statistical analysis to be carried out. The designation of individual cows to group one or two for within-pair analysis was carried out in the same way as method one. For the between-pair analysis, all the pairs of cows within treatment C were assigned to group one, and the treatment 2 pairs were assigned to group two.

The first method allowed for a larger data set to be utilised, whilst the second method enabled a between-pair analysis to be carried out without any possible effects of the different deprivation of the cows to interfere with the legitimacy of the results.

Other behaviours collected continuously were not suitable for an ANOVA, as they were either uncontinuous, or not normally distributed.

All behaviours, for both groups, for within-pairs and between-pairs, and for each method were normally distributed (as estimated by the Kolmogorov-Smirnov test, $P \geqslant 0.05$). All the data within each behaviour were homogenous (as defined by the F-Test, $P \geqslant 0.05$). For each method, an ANOVA was undertaken for each behaviour between the two groups for both within-pairs and between-pairs to calculate the error of the mean square, which provides the best estimate of the population variance (Dytham, 1999). Using this value the standard error of the difference (SED) between the two groups was calculated for each behaviour for both within-pairs and between-pairs, in each method. The coefficient of variation (CV) for both within-pairs and between-pairs for each behaviour in each method were calculated using the SED. An ANOVA was carried out to compare the within-pairs and between-pairs coefficient values for each of the behaviours analysed in method one, as these values followed a normal distribution (as estimated by the Kolmogorov-Smirnov test, $P \geqslant 0.05$) and data for comparison were homogenous (as defined by the F-Test, $P \geqslant 0.05$). A Mann-Whitney test was used to analyse the within-pairs versus between-pairs coefficient values for method two, as the within-pairs coefficient values were not normally distributed (as estimated by the Kolmogorov-Smirnov test, $P<0.05$) and were resistant to mathematical transformation. The Kolmogorov-Smirnov analysis was selected over alternative methods to determine whether the observations followed a normal distribution as it is a comparatively more lenient test towards detecting non-normality (Minitab, 2000), and the Mann-Whitney test does not make assumptions about normal distributions (Dytham, 1999).

5.4b Results

Table 5.1b shows the coefficient of variation values calculated for each of the behaviours within-pairs and between-pairs in both methods.

Table 5.1b Coefficient of variation values (%) for between-pairs (BP) and within-pairs (WP) for each behaviour in each method

	Method 1		Method 2	
	Coefficient of variation (%)		Coefficient of variation (%)	
Behaviour	BP	WP	BP	WP
Feeding	9.5	9.4	17.3	16.5
Standing	14.7	14.8	15.1	16.3
Lying	17.8	17.7	20.9	20.9
Ruminating lying	18.0	18.4	16.1	16.7
Ruminating standing	32.3	31.6	N/A	
Leg raising	23.4	23.2		

There was no difference between the within-pair and between-pair coefficients of variation for any of the behaviours when analysed using either method one or method two (P=0.98, P=0.77, respectively).

Fig. 5.2b shows the distribution of the coefficient of variation values for both within-pairs and between-pairs, for method one, and Fig. 5.3b for method two. Fig. 5.2b illustrates that the coefficient values for both within-pairs and between-pairs are very similar. The coefficient data points approximately follow a straight line. Using the second method of analysis gave rise to diminutive differences between the within-pairs and between-pairs coefficient values for three of the behaviours. These differences can be observed for standing, feeding and ruminating lying (Fig. 5.3b).

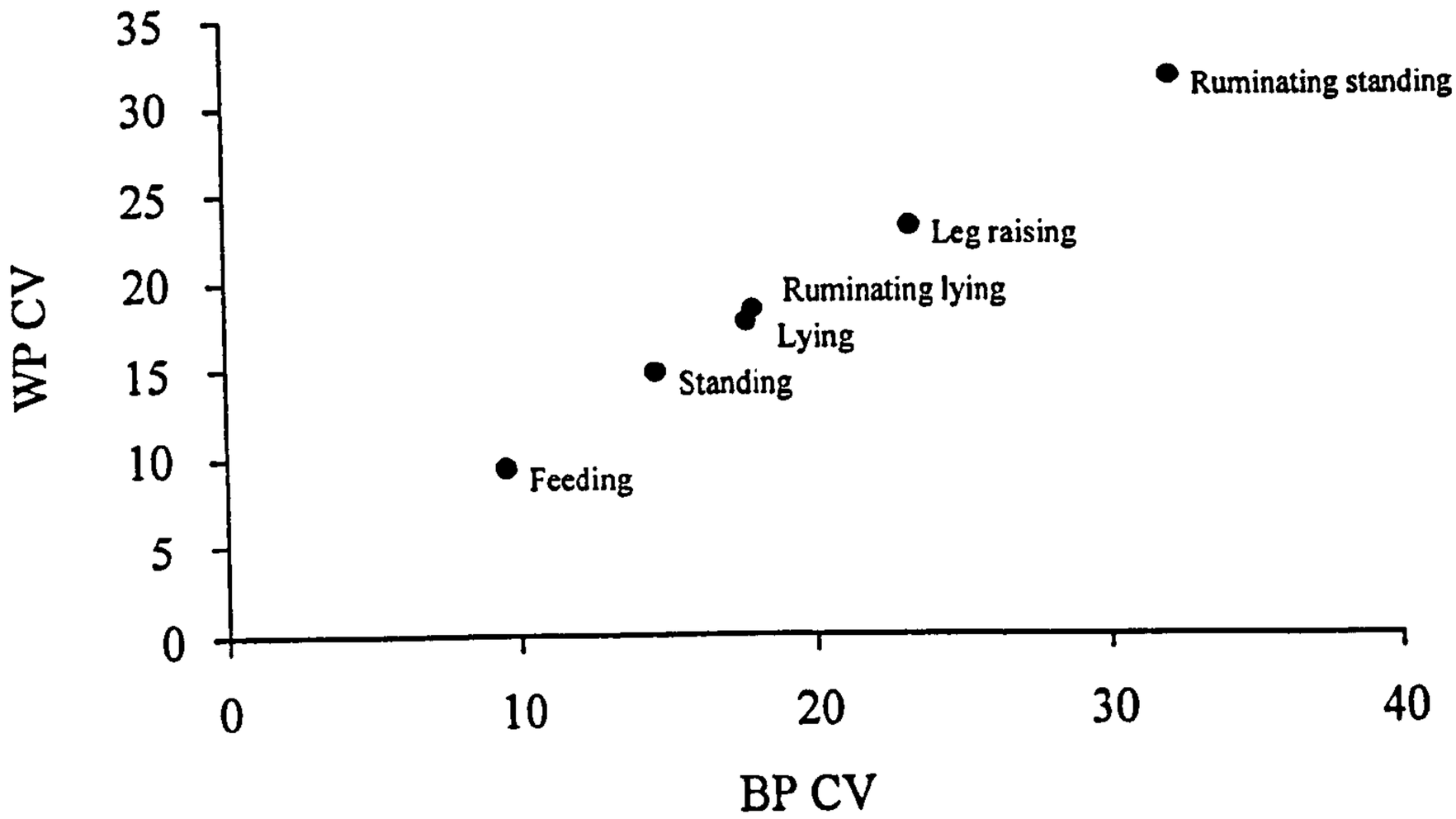


Fig. 5.2b Within-pair (WP) coefficient of variation (CV) values (%) plotted against between-pair (BP) values (%) for each behaviour in method one

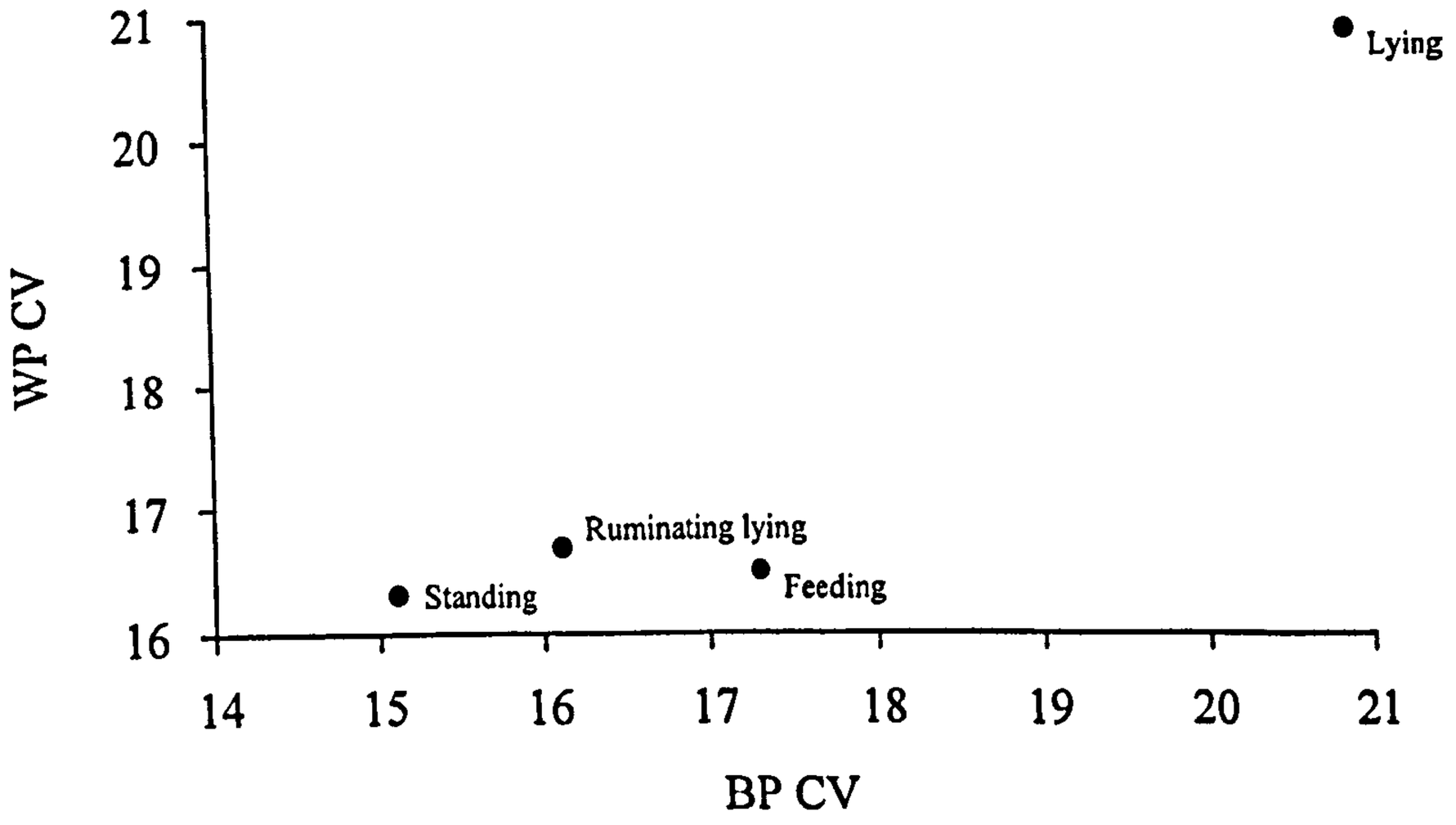


Fig. 5.3b Within-pair (WP) coefficient of variation (CV) values (%) plotted against between-pair (BP) values (%) for each behaviour in method two

For standing and ruminating lying, the within-pair variation was slightly greater than the between-pair variation. The opposite was true for feeding. None of these

differences were significant, but resulted in the observed non-linear distribution of data points when plotted (Fig. 5.3b).

5.5b Discussion

For the cows used in this study, under these conditions, the two methods of analysis revealed that there was no consistent trend for the variation in behaviour between cows within-pairs to be significantly different from that existing between-pairs. This suggests that cows within the same group behave in a similar fashion to cows not within the same group, which is convincing evidence to suggest that, for the behaviours examined, cows within groups behave independently. Supporting this, Ingvartsen *et al.* (1999) found no difference in the variance for dry matter intake between two groups of eight cows housed in stalls (0.1657 versus 0.1321).

Behaving as an individual appears logical, as certain cows within a group may be more highly motivated to perform a certain behaviour than other members, and the thwarting of a behaviour may be detrimental to the cow's welfare. For instance, a high yielding dairy cow requires a greater energy intake to sustain its level of production in comparison to a lower yielding cow (Phillips & Denne, 1988). This extra energy intake may only be achieved by increasing the amount of time spent grazing (see Chapter 3b), therefore her motivation to feed would ensure that she performed this behaviour despite the individual needs of other members within the group. The drive to satisfy such a need over maintaining group cohesion may be due to the dilution of innate survival mechanisms through domestication. In evolutionary terms, there would have been advantages in behaving as a group and synchronising certain behaviours, as performing a behaviour in isolation may have increased the chances of

being attacked by a predator in making itself more identifiable. However, domestication has bought about cows that are less fearful which may have enabled them to become less group dependent. Although such a survival method may have become weakened, it may still be present. The commencement of lying, for example, has been observed to be synchronous, but the total amount of time spent performing it remains individually dependent (Wierenga & Hopster, 1990). Schrader (2002) observed a coefficient of variation for lying time of 27.8% between 31 loose-housed dairy cows, which was measured over a three month period indicating individual behavioural consistency. Other behaviours have also been shown to vary considerably amongst group members, suggesting independence. Phillips and Denne (1988) observed cows grazing over a 24 h period, finding a between-cow coefficient of variation for grazing time of 24%, which can be considered as moderately variable. Similarly, Schrader (2002) noted a coefficient of variation between dairy cows of 40.8% for the mean time spent active (*i.e.* in locomotion or standing) per day over three months. The findings of this study can also be supported by an earlier study (Chapter 3a), which investigated the social interactions existing between housed dairy cows. This study also concluded that there was no strong evidence to support the occurrence of interdependence amongst dairy cows whilst feeding and lying.

For some of the behaviours there was a comparative difference between the within-pairs and between-pairs coefficient of variation values, between the two methods. This may have been due to the size of the data sets used. The first method used all the data collected over the entire deprivation period, *i.e.* four hours, whilst method two only examined the data collected from the first two hours of this period.

Although this is convincing evidence for the justification of the use of individual cows in data analysis, some factors should be taken into account before applying the results to alternative data sets. The results obtained may be specific to this study as they may vary with group size, the length of time the cows have been together, the behaviours studied, and the experimental conditions. The cows within this study were housed in pairs. This does not truly reflect the 'normal' on-farm situation. Cattle are social animals and typically sub-group into approximately 10-12 individuals (Phillips, 1993). A larger group size may produce a more pronounced dominance hierarchy and alter the dynamics of the group and subsequently the behaviour of the individuals. As resources were plentiful, there may have been no advantage in asserting dominance over only one other animal. Also, the pairs of cows had only been housed together, under the experimental conditions, for approximately three hours before the observations took place. This may not have been long enough for the more dominant cow of the pair to have emerged. The experimenter did not observe any dominance-establishment type behaviours occurring during this time. Cows also behave differently under extensive conditions and tend to synchronise their grazing and lying more strongly (Atkeson *et al.*, 1942). Rook and Huckle (1995) suggested the occurrence of interdependence after observing cows grazing together. Cows may synchronise their behaviour more strictly whilst outside due to increased anxiety of predatory attack, triggering an innate anti-predator mechanism. Housed cattle tend to be less synchronised in their behaviours (O'Connell *et al.*, 1987), as they may feel safer when enclosed. In this case the difference between individuals within a group of housed cattle may be the same as the difference between individuals within different, non-interacting, groups of grazing cattle. It is also likely that the synchronisation of behaviour whilst outside is predominantly influenced by environmental factors, such

as photoperiod, weather and management practices, rather than allelomimicry (Phillips, 1998).

To fully examine the conclusiveness of these results, an examination of interdependence should take place using several larger, non-interacting groups of grazing dairy cows. Measurements could be taken of the time spent in various behaviours for each cow over a given time period, after dominance hierarchies have been established. The amount of variation existing between individuals within groups could be compared with that of individuals not within the same group, to determine whether cows within groups were more similar in their performance of the observed behaviours, which would be indicative of interdependence. An alternative method would be, for example, to have five non-interacting groups of five grazing dairy cows and record the behaviour of all cows in all groups, after dominance establishment. Each member of each group would then be re-allocated to form five new groups of individuals that have not been together previously, and measure their behaviour as before. This procedure could be repeated up to five times. This data could be used to determine whether the behaviour of each cow altered significantly between groups. If cows behave truly independently, then there should be no significant change in their behaviour between groups. Conversely, if there were group effects, their behaviour would be expected to change between groups.

In conclusion, on the basis of these results, the use of individual cows as replicates in the statistical analysis of their behaviour would appear to be legitimate. This makes current data manipulation, which could lead to reduced sensitivity and misleading results, unnecessary.

6a. The effect of short-term lying and feeding deprivation on the behaviour of lactating dairy cows

6.1a Introduction

This study is a continuation from the theme of the previous lying deprivation experiment (Chapter 5a). To prevent repetition, the reader is referred to the introduction to Chapter 5a (Section 5.1a).

Grazing high yielding dairy cows are both motivated to feed and rest simultaneously, as a direct motivational conflict has been observed between these behaviours, whereby one is pursued at the expense of the other (Veris *et al.*, 1980; Metz, 1985; Fuerst-Watl *et al.*, 1999; Fregonesi & Leaver, 2001). This conflict is mainly due to the feed intake capacity of dairy cows not having increased to the same extent as their milk production (Kamphues, 1998). This results in dairy cows, especially high yielding cows, being unable to consume sufficient energy to satisfy their appetite. It is probably grazing cows that are at an increased risk of suffering from hunger than those cows managed under intensive conditions, as grazed herbage can only be consumed at approximately 15-20g DM min^{-1} compared with 40g DM min^{-1} for silage (Phillips, 2000b). Therefore, extensively-managed high yielding cows have to extend their grazing period to consume sufficient amounts of herbage to achieve satiety (Phillips & Denne, 1988). This is probably achieved by grazing earlier in the morning (Phillips & Rind, 2002) and later at night, in comparison to their lower yielding counterparts (Phillips & Denne, 1988). Such a coping strategy results in the cow having to neglect the expression of other important behaviours such as lying down to rest (Chapter 3b & 5a). As cattle demonstrate a strong motivation to lie down (Metz, 1985), a reduction in this behaviour is likely to compromise their welfare.

The current experiment was conducted in addition to the initial lying deprivation study for two reasons. Firstly, in this study, the cows were deprived of feeding as well as lying. In the previous study the cows were observed feeding during the deprivation period. This allowed the cows to re-schedule their behaviour by resting during those times when they would have been feeding, post-deprivation. This strategy would not be available to the high yielding dairy cow. Therefore, if the cows are prevented from lying and feeding simultaneously, this would ensure that at the end of the deprivation period the cows would probably be motivated to perform both behaviours. This may be experienced by the grazing high yielding dairy cow (Chapter 3b & 5a) (Veris *et al.*, 1980; Metz, 1985; Fuerst-Watl *et al.*, 1999; Fregonesi & Leaver, 2001). Secondly, such a comparison also makes it possible to examine the behavioural priorities of the animal. From a welfare perspective, it is important not only to know whether an animal values something or not, but also by how much. To do this, it is necessary to attach a 'cost' to a preference to determine its relative importance to an animal. To successfully measure the strength of motivation, an animal has to sacrifice something that is meaningful to it in order to obtain its desired choice. This may be food, time, effort or anything which conflicts with the animal's motivation to obtain its preferred option (Dawkins, 1983b). For example, the strength of a preference can be determined by pitting a preference against a known, valued resource. Such a method involves the animal making a choice between the given resource and another resource that it is known to value. This could be, for example, food, or in the case of social animals, companionship. Food is a useful comparator, as it is known to be an essential resource and its deprivation results in suffering in sentient beings (Dawkins, 1988; Webster,

1995). This test therefore also sets up a direct motivational conflict and asks the animal how important one resource is in comparison to the other.

The aim of the current study was to determine whether short-term deprivation of lying compromises the welfare of lactating dairy cows. The subjects were also deprived of feeding, as the study was particularly interested in examining the motivational conflict between lying and feeding observed in high yielding dairy cows. Behavioural observations were employed during the deprivation period to reveal any frustration and stress experienced by the cows and also possible coping strategies. Behavioural recordings were also taken after the deprivation period to determine the behavioural priorities of the restricted behaviours. Post-deprivation milk yields were also recorded as an indication of possible production changes.

6.2a Materials and methods

6.2.1a Animals

The experiment was conducted over 18 consecutive days, between 28th June and 15th July 2002 inclusive, using cows from the Holstein-Friesian dairy herd of Moulton College, Northampton. A group of 32 late lactation (*i.e.* due to be dried-off within approximately one month) dairy cows was identified from which 18 cows (mean of 273 days in milk) were selected, and divided into three groups (blocks) of six cows (Table 6.1a). Seven of these cows had previously been used in a lying deprivation experiment (Chapter 5a). The experimental cows had a mean milk yield of 21.8 kg d^{-1}, based on National Milk Records (NMR) taken on 17th June 2002, and a median lactation number of two (Table 6.1a).

Table 6.1a Characteristics (cow no., block, pair no., mean body condition score (BCS), lactation number, days in milk, days to parturition, and milk yield) of the experimental animals selected

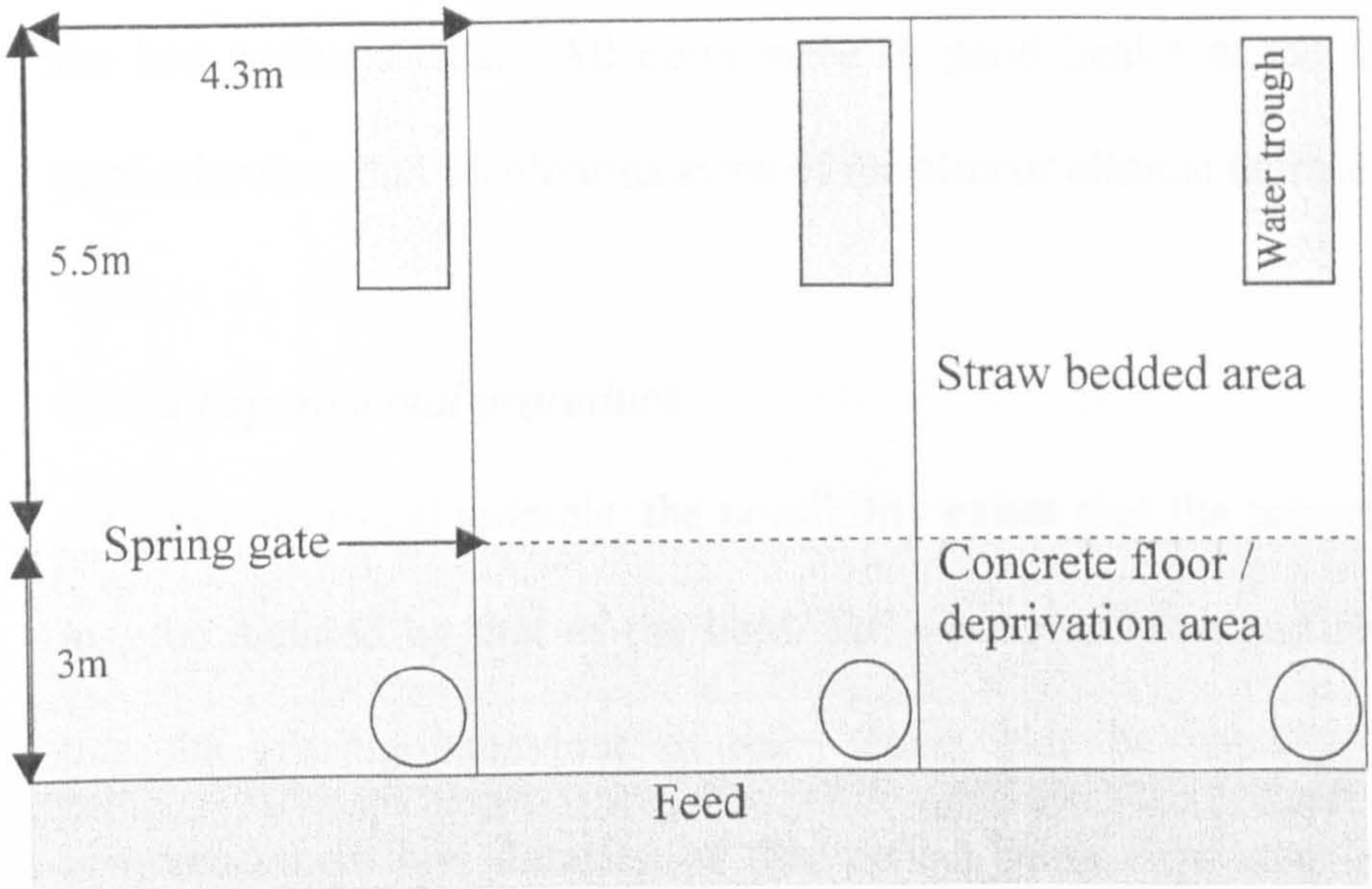
Cow Number	Block	Pair	Mean BCS	Lactation Number	Days in Milk	Days to Parturition	Milk Yield (kg d^{-1})
149	1	1	2.75	1	245	74	20.4
157	1	1	3.00	1	272	70	22.6
2	1	2	3.17	3	275	72	22.6
134	1	2	3.00	1	295	73	16.2
185	1	3	3.17	5	276	71	14.2
284	1	3	3.25	2	339	71	15.8
22	2	4	3.25	3	264	78	19.4
64	2	4	3.08	1	272	76	18.4
145	2	5	2.67	2	249	75	20.0
173	2	5	2.83	2	282	83	19.4
161	2	6	3.00	1	278	77	19.8
509	2	6	2.67	6	265	82	25.4
46	3	7	3.00	1	296	93	24.8
590	3	7	3.25	2	246	87	20.6
131	3	8	2.67	3	269	92	33.8
137	3	8	2.75	1	257	93	26.4
104	3	9	2.75	1	258	88	27.0
139	3	9	2.67	1	281	88	24.8

All cows were scored for body condition by the same assessor on a scale of one to five, to within 0.25 of a unit (Edmonson *et al.*, 1989), on three separate occasions on their first day of undergoing the experiment, and had a mean body condition score of 2.94 (Table 6.1a). All cows were due to calve in September 2002 and had a predicted mean of 80 days until parturition (Table 6.1a).

6.2.2a Experimental conditions

When the cows were not undergoing the experiment they were kept together as part of a herd of 80 low yielding cows and grazed in a 4.74 ha paddock between c. 09.00 and 05.00 h with *ad libitum* access to drinking water and an Italian ryegrass pasture (*Lolium multiflorum*) (sown 1999, composed of equal mixes of: Atalja IRG, Ligrande IRG, Solid Hybred Tetraploid IRG, and Donergo Tetraploid IRG), which was kept at the recommended height of 8-10 cm (Chamberlain & Wilkinson, 1996). From c. 05.00 to 09.00 h, the cows were housed indoors in a large, naturally lit, straw bedded building (70 x 15 m) with access to drinking water and a total mixed ration [maize silage (41.1%), second cut grass silage (27.4%), concentrate pellets (Moulton balancer, Heygates Ltd, Northampton, UK) (16.6%), wet maize gluten (Trafford gold, Heygates Ltd, Northampton, UK) (7.8%), molassed sugar beet (3.9%), lucerne pellets (2.9%) and minerals (0.3%]. Feed was delivered once per day during morning milking using a Keenan mixer wagon. Chopped straw was provided daily in appropriate amounts to keep the bed surfaces clean. During periods of darkness, artificial lighting was provided at either end of the shed. All cows were milked twice daily, from c. 05.30 to 07.00 h, and c. 16.00 to 17.30 h. A concentrate allowance of 1 kg (Grassmaster 18, Heygates Ltd, Northampton, UK) was offered in the parlour to each cow per milking.

During the experiment, the cows were housed in pairs in adjacent pens (8.5 x 4.3 x 4.2 m) (L x W x H) (Fig 6.1a) with *ad libitum* access to drinking water and a standard total mixed ration [second cut grass silage (53.5%), maize silage (30.6%), wet maize gluten (Trafford gold, Heygates Ltd, Northampton, UK) (15.3%), and minerals (0.6%)].



Circles indicate water bowls available to deprived cows during deprivation period

Fig 6.1a Plan of experimental pens

The pens were situated within a larger barn with housed cows, and were constructed from Poldenvale hurdles. Cows thus had visual, auditory, olfactory and some minimal physical contact with one another and the vision of the observer monitoring the cows' behaviour was not restricted. Feed was delivered once per day during morning milking using a Keenan mixer wagon. During periods of darkness, artificial lighting was provided overhead by six florescent tube lights to facilitate video recording of behaviour. All cows were milked twice daily, between c. 07.00 and 08.30 h, and c. 15.00 and 17.20h, and milking lasted a mean time of 57 and 69 min per block of

cows, respectively. For the final period, afternoon milking times were brought forward, cows were milked between c. 14.30 and 17.10 h, and milking lasted a mean time of 53 min per block of cows. A concentrate allowance of 1 kg (Grassmaster 18, Heygates Ltd, Northampton, UK) was offered in the parlour to each cow per milking. Faeces were removed manually from the bedded and concrete areas twice daily during milking times. Clean straw was also provided daily, in appropriate amounts to keep the bed surfaces clean. All cows were in good health at the start of the study, in particular they had no obvious signs of mastitis or clinical lameness.

6.2.3a Experimental procedure

As cows are social animals, the possibility exists that the behaviour of an individual may be dictated by that of the herd. For example, Rook and Huckle (1995) believe that the grazing behaviour of dairy cows may be socially facilitated, with the commencement and duration of this period being dependent on a few individuals within the group. Various researchers have also reported on the synchronisation of other behaviours, such as lying (Atkeson *et al.*, 1942; Schmisseur *et al.*, 1966; O'Connell *et al.*, 1987). However, although this behaviour may be synchronous, it is not thought to necessarily affect total lying time, but more the commencement of lying (Wierenga & Hopster, 1990). The experimenters therefore attempted to cater for the possibility of such interdependence by housing the subjects in pairs and for a period of only 48 h at a time. If the cows were housed singly, this may have affected their welfare and consequently their behaviour (Munksgaard & Simonsen, 1996; Watts & Stookey, 2000), and a larger group size may have succumbed to the effects of allelomimicry. If the cows had been housed together for a longer period, the more

dominant cow of the pair may have emerged and started to alter the behaviour of the other cow.

6.2.4 Cow management

Each block of cows underwent the experimental procedure three times. Each procedure lasted 48 h and then the cows were returned back to the herd during morning milking when the next block of cows was collected. As blocks of cows were tested consecutively, each block spent four days back with the herd between tests. Each block of six cows was initially selected from the group of 32 cows immediately following morning milking (c. 07.20 h) on their first day of undergoing the experiment. Cows were selected according to their order of exit out of the parlour, as long as they had not previously been used in the experiment. The first six cows were in block one, the next six in block two and the third six in block three. The cows were divided into pairs at random and numbered consecutively, starting with the first block of cows (Table 6.1a). To avoid possible confounding effects of pen, in each period each pair of cows (and therefore treatment) was randomly (determined by coin tossing) allocated to an experimental pen (Table 6.2a).

Table 6.2a Allocation of pairs to pens for each block of cows in each period

		Pair number		
Block	Period	Pen 1	Pen 2	Pen 3
1	1	3	1	2
	2	1	2	3
	3	3	2	1
2	1	4	5	6
	2	5	4	6
	3	4	5	6
3	1	7	8	9
	2	8	7	9
	3	9	7	8

Each pair was subjected to a different treatment in each period, following an unbalanced Latin square design (Patterson & Lucas, 1962) to avoid possible order of treatment effects (Table 6.3a). As there were only six possible combinations, pairs in block three followed the same order of treatments as pairs in block one.

Table 6.3a Unbalanced Latin square design showing order of treatments for each pair in each block for each period (C = control; 2 = 2 h deprivation; 4 = 4 h deprivation)

	Block 1			Block 2			Block 3		
	Pair			Pair			Pair		
Period	1	2	3	4	5	6	7	8	9
1	C	2	4	C	2	4	4	C	2
2	2	4	C	4	C	2	C	2	4
3	4	C	2	2	4	C	2	4	C

The three treatments were control (Treatment C) (no lying or feed deprivation), 2 h of lying and feed deprivation (Treatment 2), and 4 h of lying and feed deprivation (Treatment 4). On each occasion, each block of experimental cows had approximately three hours to become accustomed, or re-accustomed, to the pens and their pen mate before behavioural observations commenced. If the paired cows natural markings or features were too similar that they could not be easily distinguished, one cow of the pair was marked with an 'X' on either side of her body using a black or white marker spray (Ritchey stock marker). The same cow was always marked. Lying deprivation was achieved by securing the cows into the deprivation area using a spring gate (Rutland electric fence 17-120) to prevent access to the bedded area (Fig. 6.1a). It was not necessary to electrify the fence. The 4 h deprivation period commenced at 10.15 h and the 2 h period started at 12.15 h. Both deprivation periods therefore terminated at 14.15 h, at which time the spring gates were removed and the cows gained access to

the bedded area. The relative discomfort of the concrete floor, the presence of a small amount of urine and faeces, and being in a confined area appeared to discourage the cows from lying in the deprivation area. An observer was present throughout the deprivation period to ensure the cows did not lie down. The timing of the deprivation periods were considered most appropriate as a previous study had revealed that the cows were motivated to lie during these times. In addition, they did not interfere with farm management practices, such as milking schedules. The timing and length of the deprivation periods were also selected to be as practically relevant to the real situation faced by high yielding dairy cows as possible, *i.e.* the subjects were deprived during the day, as this is when high yielding cows spend extended periods standing in order to continue grazing (Chapter 3b). The total mixed ration was only available to the treatment C cows during the deprivation period. Feed for the treatment 2 and 4 cows was withdrawn during deprivation. Water was available *ad libitum* to all of the experimental animals at all times.

6.2.5a Milk yield recording

Milk yield was recorded on four consecutive occasions for each cow in each period, whilst undergoing the trial, to examine any possible effects of lying and feed deprivation on milk production. Recordings were taken at afternoon milking on day one (the deprivation day) (first milking), morning and afternoon milking on day two (second and third milking), and morning milking on day three (fourth milking), when the cows were returned back to the herd. Records were taken by two observers, who had been trained to record milk yields by the NMR data collector. NMR taken on 17th June were used as control recordings for the cows.

6.2.6a Behavioural observations during deprivation

The behaviour of each cow was recorded during the deprivation period by two observers. A single observer recorded all cows at any one time. Observers were rotated sequentially. Both observers were trained in the behavioural recording methods employed to reduce interobserver error. The cows were always observed in the same order.

6.2.6.1a Continuous recordings

The behavioural activities of all animals were recorded using continuous recording (Martin & Bateson, 1995), which measured the frequency and/or duration of each behaviour pattern as it occurred. Bouts were separated by the cow showing at least 1 s of another behaviour. The behaviours were selected to provide possible indicators of frustration and fatigue. A new behavioural recording sheet was used each hour to examine whether certain behaviours changed in frequency over the deprivation period. The behaviours were previously determined by spending one eight hour period with the cows, which included observing some cows continuously standing for up to two hours during milking times. The 24 mutually exclusive ethological characteristics selected for analysis are represented in Table 6.4a.

Table 6.4a Behaviours recorded continuously by frequency (a) and duration (b) during the deprivation period

(a) Behaviours recorded by frequency

Behavioural variable	Definition
Leg raising	Lifting hoof and replacing on same spot without forward momentum (Houpt *et al.*, 2001)
Repositioning	Moving all four legs slowly forward one at a time in a synchronised manner covering less than one body length in distance. Only performed by deprived cows due to confined area.
Walking	Moving all four legs slowly forward one at a time in a synchronised manner covering greater than one body length in distance.
Butting	Use of head by one cow to physically displace the other (Phillips, 2001)

Pushing	Deliberate use of a part of the body, other than the head, to physically displace the other cow (Phillips, 2001)
Threat	Where the head was swung in the direction of the other cow, who took avoidance action (Phillips, 2001)
Vocalising	Cow making sound with mouth either open or closed (Sandem *et al.*, 2002)
Head swing	Swinging of head either upwards or to the side
Feed tossing	Tossing feed into the air with nose
Lying	Body resting on floor (Munksgaard *et al.*, 1999). Only applies to deprived cows.
Weight shifting	Displacing weight from one side of the body to the other by either relaxing a leg or shuffling the legs
Grooming self	Self licking any part of the body
Grooming other	Licking any part of the body of the other cow
Sniff ground	Sniffing the ground
Nose ground	Nosing the ground
Lick ground	Licking the ground
Lick housing*	Licking the housing

(b) Behaviours recorded by duration

Behavioural variable	Definition
Leaning against housing*	Leaning of body against housing
Rubbing body against other	Rubbing of any part of the body or head against the other cow
Rubbing body against housing*	Rubbing of any part of body, except the head, against housing
Rubbing head against housing*	Rubbing of head against housing
Sniff housing*	Sniffing the housing
Drinking	Drinking from water trough/bowl
Sleeping	Lying down with neck relaxed and eyes closed

*Housing refers to gates, walls, feeding barrier and water trough

6.2.6.2a Instantaneous recordings

The behavioural activities of all animals were recorded using instantaneous scan sampling (fixed interval time point sampling or point sampling) (Martin & Bateson, 1995) at 5 min intervals. These time intervals have been shown to provide an accurate analysis of the major behavioural activities such as feeding (Hull *et al.*, 1960). The five mutually exclusive ethological characteristics selected for analysis were: Feeding, standing, ruminating standing, ruminating lying and lying (Table 6.5a). For each individual cow the total time spent in each activity was estimated by assuming that they had been performing the behaviour for the remainder of the 5 min period and

multiplying the number of recordings of each behaviour by the number of scans in the four hour period.

Table 6.5a Behavioural categories recorded instantaneously during the deprivation period and from the videotapes post-deprivation

Behavioural variable	Definition
Feeding	The animal has food in the mouth and/or chews (Munksgaard *et al.*, 1999)
Ruminating standing*	Chewing regurgitated cud whilst standing
Ruminating lying*	Chewing regurgitated cud whilst lying
Standing	Standing (excluding feeding and ruminating)
Lying	Body resting on floor (excluding lying ruminating) (Munksgaard *et al.*, 1999)

*Only recorded during the deprivation period

6.2.7a Behavioural observations post-deprivation

The behaviour of each cow post-deprivation was quantified by the same observer.

6.2.7.1a Lying position and changes in posture

For 45 min after the deprivation period, the lying position (*i.e.* either lateral or sternal) and the number of changes in posture were recorded for each cow that was observed lying. If a cow was laterally recumbent then her lying side, *i.e.* left or right, was also noted. Observations were only taken for the first two periods, as afternoon milking times were brought forward in the third period and only allowed 15 min between the end of the experiment and milking.

6.2.7.2a Post-deprivation video observations

Continuous time-lapse (2.60 mm s^{-1}, *i.e.* one 3 h tape for every 24 h) monochrome video recordings commenced immediately after the deprivation period for the following 41 h spent in the pen for each group. A 41 h period was selected as it was

anticipated, from the literature, to provide ample time for any behavioural effects of the deprivation to be demonstrated (Metz, 1985). Tapes were changed whilst the cows were being milked in the afternoon. The videotapes were analysed by instantaneous scan sampling (Martin & Bateson, 1995) at 5 min intervals. Observations were conducted during all periods except when the cows were being milked. The following behaviours were recorded: Feeding, standing and lying. Table 6.5a describes the mutually exclusive behavioural categories quantified. For each individual cow the total time spent in each activity was estimated by assuming that they had been performing the behaviour for the remainder of the 5 min period and multiplying the number of recordings of each behaviour by the number of scans in the 41 h period. The videocassette recorder was a VHS Mitsubishi HS-1024EB. The camera was a Sanyo 3372 fitted with a varifocal (3.5 – 8 mm) lens.

6.3a Statistical analyses

All statistical analyses were performed using the 13th release of the Minitab statistical programme (Minitab, 2000). A probability value was considered significant when $P \leqslant 0.05$. A probability value between 0.05 and 0.1 was referred to as a trend or tendency. Individual cows were used as replicates for all statistical analyses (see Chapter 6b for justification of this procedure). Normal distributions were defined by the Anderson-Darling test ($P \geqslant 0.05$), and tests for homogeneity of variance were defined by Bartlett's test if normally distributed, or Levene's if not normal ($P \geqslant 0.05$). The F-test replaced Bartlett's when there were only two levels for comparison. For each measure, the General Linear Model (GLM) was selected to determine differences between two or more data sets, providing that the values were normally distributed and the data sets to be compared had equal variance. The GLM was selected to adjust for possible differences between replicates and periods and to determine the significance of the interactions between the factors. It was not possible to determine the effects of previous treatment by including it within the model, as this factor was confounded with period, *i.e.* all cows in period one had not previously been subjected to a treatment. Thus, the effect of previous treatment on each measure was examined separately using a one-way analysis of variance (ANOVA), provided the data were suitable for parametric analysis, or the Kruskall-Wallis test, if not. The Friedman test was selected as a non-parametric alternative to the GLM, which allowed for data to be blocked for replicate, therefore taking into account any variation existing between individual cows. For measures with greater than 50% zero values, for one or more data sets, the 1-Sample Sign test was used to determine the true median value for each data set (Snedcor & Cochrane, 1978). More than one test may have been conducted on comparative data sets.

6.3.1a Milk yield recordings

All data, for each treatment, taken during the first and second milkings, and when combined, and during the third and fourth milkings, and when combined, and when a mean was taken for both recordings, were normally distributed. All data for each treatment combination were homogenous.

For the mean morning milk yield, the effect of deprivation on milk yield was not clear from an examination of individual treatment differences. Thus, data for the cows when in treatments 2 and 4 were combined to examine a control versus deprivation treatment effect. When the milk yield data were combined in this way, all milk recordings were normally distributed and data for each treatment combination were homogenous.

6.3.2a Continuous recordings

As the behaviours observed were mutually exclusive, it was possible to combine behaviours to form new behavioural categories. The individually-recorded behaviours, which were combined to form new behavioural categories are listed in the statistical analyses section of Chapter 5a (Table 5.5a). Justification for the grouping and labelling of certain behaviours can also be found in this section.

The following behaviours were not observed: Feed tossing, leaning against housing, licking ground, lying, pushing, rubbing body against housing, rubbing body against other, grooming other, and threat. For all treatments, licking housing, sniffing housing, sleeping and vocalising had too few observations for any useful statistical

analysis to be carried out. When in treatment C the cows were rarely observed butting and nosing the ground, thus there was insufficient data to conduct any useful statistical analysis. All the data recorded for each behaviour were discrete and had low frequencies (less than 29 possible values) and thus were not suitable to analyse parametrically.

Each behavioural variable was first tested for differences between hours, for each treatment. When in treatment C, all behaviours, except leg raising, head swinging, the frequency of, and number of licks whilst grooming self, walking, body care, licking, shifting, restlessness, stress, and frustration had greater than 50% zero values for one or more hours. When in treatment 2, repositioning, weight shifting, butting, the frequency of, and number of licks whilst grooming self, the frequency and duration of rubbing head against housing, sniffing ground, the frequency and duration of drinking and nosing ground had greater than 50% zero values for one or more hours. When in treatment 4, butting, the frequency of, and number of licks whilst grooming self, the frequency and duration of rubbing head against housing, and the frequency and duration of drinking had greater than 50% zero values for one or more hours. Those behaviours with less than 50% zero values for two or more hours were as follows: Repositioning (treatment 2), weight shifting (treatment 2), butting (treatment 4), the frequency of, and number of licks whilst grooming self (all treatments), the frequency and time spent rubbing head against housing (treatment 4), sniffing ground (treatment 2), and nosing ground (treatment 2). When the cows were in treatment 2, data for shifting (hours three and four) and stress (hours three and four) followed normal distributions. When in treatment 4, data for leg raising (hours one, two and four) shifting (hours two and four), restlessness (hours two, three and four) and stress

(hours two, three and four) followed normal distributions. For the first hour of leg raising it was necessary to use logarithms of the original data to achieve a normal distribution. The variances were homogenous between all hours for each normally distributed behaviour.

Each behavioural variable was then tested for treatment differences for each hour and for the average frequency/duration of the activity over the entire four hour deprivation period. The data for the average frequency/duration were calculated by dividing the total frequency/duration a cow was observed performing a behaviour over the deprivation period by four. For each hour, repositioning, weight shifting, butting, the frequency and duration of rubbing head against housing, sniffing ground, the frequency and duration of drinking and nosing ground had greater than 50% zero values for at least one treatment. The frequency and duration of self grooming (hours three and four only), sniffing and housing interaction (hours two, three and four for both) had greater than 50% zero values for at least one treatment. All behaviours for the average frequency/duration over the entire deprivation period, except butting, the frequency and time spent drinking, and nosing ground had less than 50% zero values for each treatment recorded performing the behaviour. Those behaviours with less than 50% zero values for two or more of the treatments were as follows: Repositioning, weight shifting, nosing ground (all hours three and four), sniffing ground (hours one, three and four), the frequency and time spent drinking (hour one and average data for all hours), body care (hour three), sniffing, housing interaction (hours two, three and four for both), butting and nosing ground (average data for all hours for both behaviours). For shifting (hour four) and stress (hours three and four) cows undergoing treatments two and three had data following a normal distribution.

Spearman's rank order correlation coefficients were determined between each behaviour (using the average frequency/duration of a behaviour over the entire deprivation period) and milk yield (NMR data), the number of days in milk, the number of days to parturition, body condition score and lactation number, for each treatment. The Spearman's rank order correlation was selected over the Pearson's correlation as the data was not continuous or normally distributed (Dytham, 1999). A linear regression analysis was performed on statistically significant ($P \leqslant 0.05$) correlations. For significant ($P \leqslant 0.05$) regressions, there was no evidence for non-linearity. Only those behaviours significantly correlated with at least one treatment are shown and discussed in the results section.

6.3.3a Instantaneous recordings

As the behaviours observed were mutually exclusive, it was possible to combine behaviours to form new behavioural categories. The three new behavioural categories examined, and the individual behaviours combined to form them, are shown in the results section (Table 6.20a).

Each behaviour was tested for differences between hours, for each treatment, and between treatments, for each hour. When the cows were in treatment C, all behaviours, for each hour, followed a normal distribution except feeding (hour one), standing (hours two, three and four), lying (all hours), standing ruminating (all hours), lying ruminating (hours one, two and four), all standing behaviours, all lying behaviours and all standing behaviours including feeding (hours two and four for all behaviours). For standing ruminating, data for hours two and four had greater than

50% zero values. When the cows were in treatment 2, all behaviours for each hour were normally distributed except feeding (hour two), lying, standing ruminating, lying ruminating (hours one and two for all behaviours), all lying, and all standing including feeding (hour two for both). The data for lying in the first hour had greater than 50% zero values. When the cows were in treatment 4, all behaviours for each hour were normally distributed. For all treatments, all non-normal data were resistant to mathematical transformation to normalisation except for the fourth hour for standing and for all lying behaviours (both treatment C), which were transformed using the square root of the original data. For each treatment, the data for the total time spent in a behaviour, for all four hours combined, followed normal distributions except standing ruminating (treatment C), lying, all lying behaviours and all standing behaviours including feeding (all treatment 2). Data for these behaviours, except all lying behaviours, were mathematically transformed to normality using logarithms of the original data. All lying behaviours was resistant to mathematical transformation to normalisation.

Pearson's correlation coefficients were determined between each behaviour (using the data for the total time spent in a behaviour for all four hours) and production variables (see continuous recordings correlations for variables), for each treatment. A linear regression analysis was performed on statistically significant ($P \leqslant 0.05$) correlations. There was no evidence for non-linearity for significant ($P \leqslant 0.05$) regressions. For those behaviours significantly ($P \leqslant 0.05$) correlated with a production variable, the GLM analysis was initially conducted using the production variable as a covariate. When using milk yield, body condition score and the number of days to parturition as covariates, this did not improve the significance of treatment on ruminating standing,

ruminating lying and all standing including feeding behaviours and therefore the analysis without the covariates was used.

6.3.4a Lying position and changes in posture post-deprivation

There were too few observations to carry out any useful statistical analyses on the data for lying position and the number of changes in posture. For the total number of cows observed lying during each treatment a Chi-square test of association was employed to determine the effect of treatment on this behaviour. This test makes no assumptions about the form of the data and is suited for analysis of frequencies (Dytham, 1999). None of the expected values were less than five. For those cows not observed lying during this period the time spent feeding was calculated for each cow and tested for treatment differences. The data for the time spent feeding for the cows in each treatment were not normally distributed and were resistant to mathematical transformation to normalisation.

6.3.5a Post-deprivation video observations

The mean time spent in each behaviour per hour for each treatment was calculated and displayed graphically to determine whether there were any obvious differences between treatments in the behaviours performed over time. To ensure equal observation times for all cows in each block per hour, data were omitted so that all blocks had the same start and finish times for each milking. This resulted in data being omitted for hours beginning at 14.20, 15.20 and 16.20 on day one and two, and 07.20 on day two, *i.e.* hours 1, 2, 3, 18, 25, 26 and 27. Also, 5 and 15 min of data were discounted for hours beginning at 17.20 (hour 4) and 08.20 (hour 19)

respectively, and 10 min for hours 06.20 on both days (hours 17 and 41). The graphs revealed that eight hour periods would best suit the data for further exploration.

The mean time spent in each behaviour per eight hours for the 41 h period, the entire 41 h period (hours 1 to 41), and the entire 41 h period including the four hour deprivation period (hours - 4 to 41) was calculated for each treatment. Milking times were made equal for all blocks by discounting any data recorded immediately post-milking, thus ensuring all blocks were observed for the same amount of time. Data collected post-milking was discounted rather than that collected prior to milking, as the cows were considered more likely to be unsettled during this period. Data for each block of cows were discounted to equal the longest milking time for a block of cows within that milking session. The longest first, second and third milking session was 80, 85 and 100 min, respectively. These milkings took place between hours 14.30 to 17.20 (hours 2–4), 07.00 to 08.30 (hours 17–19) and 14.10 to 17.10 (hours 24-27), respectively. The fourth milking took place when the cows were returned to the herd, therefore did not affect the total observation time between blocks of cows. All data for between treatment comparisons, for each behaviour, in each time period, had equal variance. When the cows were in treatment C, all behaviours for each time period followed a normal distribution, except standing (hours 1-8), feeding (hours 9-16) and lying (hours 33-40). When the cows were in treatment 2, all behaviours for each time period were normally distributed, except feeding (hours 1-8 and 9-16), standing (hours 9-16) and lying (hours 1-41). When in treatment 4, all behaviours for each time period were normally distributed, except feeding (hours 9-16), standing (hours1-8, 9-16, 17-24, 25-32, 1-41 and - 4-41) and lying (hours 1-8, 9-16, 1-41 and - 4-41). For all treatments data for feeding in hours 9-16 had greater than 50% zero values. Non-

normal data for lying (treatments 2 and 4 for hours 1-8 and 1-41, and treatment 4 for hours – 4-41) were mathematically transformed by squaring the original data to obtain normal distributions. The data for feeding behaviour for the cows when in treatment 2 (hours 1-8) were transformed to $\log_{10}$ to obtain a normal distribution. All remaining non-normal data were resistant to mathematical transformation to normality. The correlation and regression analyses conducted followed that specified for the instantaneous data (section 6.3.3a). There was no evidence for non-linearity for significant ($P \leqslant 0.05$) regressions. For those behaviours significantly ($P \leqslant 0.05$) correlated with a production variable, the GLM analysis was initially conducted using the production variable as a covariate. When using the number of days to parturition and milk yield as a covariate, this did not improve the significance of treatment on standing and feeding, respectively, and therefore the analysis without the covariate was used. Data for the mean time spent in a behaviour during the first eight hours was used to determine the Pearson's correlation coefficients.

The latency to, and duration of, the first lying and feeding bout immediately following the first milking post-deprivation, was calculated for each treatment. Data for the latency to lie and duration of feeding when the cows were in treatments C and 4 were not normally distributed and were not improved by mathematical transformation. As the majority of the cows during each of the three treatments chose to feed immediately post-milking, the latency to feed was zero, thus there was too little data to conduct any useful statistical analysis. Data for the duration of lying when the cows were in treatment C were transformed to $\log_{10}$ to achieve a normal distribution.

6.4a Results

6.4.1a Health status of animals during the study

All cows maintained good health for the duration of the study, *i.e.* they had no obvious signs of mastitis or clinical lameness.

6.4.2a Milk yield recordings

6.4.2.1a Post-deprivation milk yields

There was a tendency for cows when in treatments 2 and 4 to have lower mean milk yields compared to when in treatment C, during the second milking post-deprivation (P=0.06) (Table 6.6a(i)). There was no significant difference in mean milk yields between treatments during the first milking post-deprivation or for the first two milkings when the data were combined (Table 6.6a(i)).

There was no significant treatment effect on mean milk yields between cows during the third or fourth milking or for the total of these two recordings (Table 6.6a(ii)).

When the cows were in treatment 2 they had a lower combined morning milk yield compared to when in treatment C (P=0.04) (Table 6.6a(iii)). The SED value suggests that there was no significant difference between combined morning milk yields for the cows when in treatment 4 compared to treatments 2 or C. The average mean milk yield over the two days for cows undergoing all three treatments was similar for the combined afternoon measurements (P=0.49) and the total of the combined morning and afternoon values (P=0.15) (Table 6.6a(iii)).

Table 6.6a Mean post-deprivation milk yield values (kg) for all the experimental cows used in the control (C), 2 h (2) and 4 h (4) treatments:

(i) The first (pm) and second (am) milkings, and their total (pm+am)

Milking	Treatment			SED ±	P-value
	C	2	4		
First (pm)	6.1	6.1	6.0	0.29	0.99
Second (am)	12.4	11.5	12.0	0.36	0.06
Total (pm + am)	18.4	17.6	18.0	0.53	0.27

(ii) The third (pm) and final (am) milkings, and their total (pm+am)

Milking	Treatment			SED ±	P-value
	C	2	4		
Third (pm)	8.2	7.6	7.5	0.43	0.26
Fourth (am)	12.8	12.1	12.6	0.46	0.25
Total (pm + am)	21.0	19.7	20.1	0.74	0.22

(iii) The combined afternoon (pm) (1st and 3rd milking) and morning (am) (2nd and 4th milking) recordings, and the mean for all four occasions (pm+am)

Milking	Treatment			SED ±	P-value
	C	2	4		
pm	7.1	6.9	6.8	0.31	0.49
am	12.6	11.8	12.3	0.31	**0.04**
pm + am	19.7	18.6	19.1	0.54	0.15

(iv) The combined afternoon (pm) and morning (am) recordings, and the mean for all four occasions (pm+am) for the control treatment (C) and the 2 h and 4 h treatments when combined (2+4)

Milking	Treatment		SED ±	P-value
	C	2+4		
pm	7.1	6.8	0.30	0.24
am	12.6	12.0	0.32	0.06
pm + am	19.7	18.9	0.54	0.08

There was a tendency for the combined morning milk yields and the total of the combined afternoon and morning milk yields to be lower for the cows when undergoing the deprivation treatments (*i.e.* treatments 2 and 4 combined) compared to when in treatment C (P=0.06; P=0.08, respectively) (Table 6.6a(iv)). There was no significant difference between milk yields for cows when undergoing the deprivation treatments compared to treatment C for the combined afternoon recordings (Table 6.6a(iv)).

Period had a significant effect on milk yield. Mean milk yields were significantly higher in period one compared to periods two and three for the combined morning and afternoon values (21.6, 18.4 & 17.5 kg d^{-1}, respectively. SED 1.72, P=0.05). Previous treatment had no significant effect on milk yield for any of the measures (P>0.10).

6.4.3a Behavioural observations taken during the deprivation period

No cow from either of the deprivation groups attempted to lie down during actual deprivation.

6.4.3.1a Continuous recordings

Each sub-table (1 to 22) in Table 6.7a shows the significance of the differences between values for each hour (by row) and for each treatment (by column), for a specific behaviour.

Leg raising did not significantly differ between hours for the cows when in treatments C and 4 (Table 6.7a1). There was a significant incidence of leg raising during deprivation for the cows when in treatment 2. For each hour, except the second, and

over the entire deprivation period, leg raising increased significantly between treatments with increasing deprivation length (Table 6.7a1). In the second hour, leg raising was significantly greater for the cows when in treatment 4 compared to treatments C and 2, which had the same frequency.

Table 6.7a The average frequency (no. h^{-1}), or duration (sec h^{-1}), that each behaviour was performed per hour of the deprivation period, and the average per hour for all four hours, for all of the experimental cows in the control (C), 2 h (2) and 4 h (4) treatments

1. Leg raising (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	2.0	2.0	2.0	3.0	0.94	2.0
2	3.5	2.0	6.0	7.5	**<0.01**	4.4
4 [(m)]	8.2 9.0	8.6 8.0	- 8.5	7.3 8.5	(1, 2&4)[1] 0.46 0.43	8.3
P-value between treatments	**<0.01**	**0.01**	**0.01**	**0.01**		**<0.01**

[m]mean values on top row

2. Repositioning (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	-	-	-	-	-	-
2	-	-	5.0	7.0	0.47	3.5
4	6.0	8.0	7.0	6.0	0.40	7.0
P-value between treatments	-	-	0.80	0.83		**<0.01**

3. Weight shifting (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}0^{c}$	$_{b}0\text{-}1^{b}$	$_{b}0\text{-}1^{b}$	$_{b}1.0^{a}$	-	0.3
2	$_{b}0^{c}$	$_{b}0\text{-}1^{b}$	$_{a}2.5^{a}$	$_{a}3.5^{a}$	(3&4)[1] 0.62	1.5
4	$_{a}2.0$	$_{a}4.0$	$_{a}3.5$	$_{a}5.0$	0.44	4.8
P-value between treatments	-	-	(2&4)[1] 0.47	(2&4)[1] 0.47		**<0.01**

4. Shifting* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	2.0	2.0	3.0	4.0	0.76	2.9
2 [m]	- 4.0	- 3.0	23.2 19.0	22.7 20.0	(3&4)[1] 0.87 **<0.01**	12.3
4 [m]	- 18.5	21.7 18.0	- 19.5	22.7 20.0	(2&4)[1] 0.45 0.48	18.1
P-value between treatments	**<0.01**	**<0.01**	**<0.01**	**<0.01** (2&4)[1] 0.99		**<0.01**

*Leg raising + repositioning + weight shifting; [m]mean values on top row

5. Head swinging (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	2.0	2.0	1.5	1.5	0.49	1.6
2	2.0	2.0	2.0	2.0	0.73	2.4
4	2.5	4.0	2.0	2.5	0.07	3.5
P-value between treatments	0.42	**0.04**	0.37	0.81		0.68

6. Restlessness* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	4.5	4.5	5.0	6.5	0.78	5.6
2	7.5	5.0	20.0	21.5	**<0.01**	14.3
4 [m]	- 20.0	28.1 22.5	25.5 21.0	25.6 24.5	(2,3&4)[1] 0.40 0.75	23.1
P-value between treatments	**<0.01**	**<0.01**	**<0.01**	**<0.01**		**<0.01**

*Leg raising + repositioning + weight shifting + head swinging; [m]mean values on top row

7. Nose ground (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{c}0^{a}$	$_{b}0^{a}$	$_{b}0^{a}$	$_{b}0^{a}$	-	$_{c}0$
2	$_{b}0\text{-}1^{b}$	$_{b}0^{c}$	$_{a}1.0^{a}$	$_{a}1.0^{a}$	(3&4)[1] 0.41	$_{b}0.6$
4	$_{a}4.0$	$_{a}3.0$	$_{a}2.0$	$_{a}2.5$	0.14	$_{a}2.8$
P-value between treatments	-	-	(2&4)[1] 0.80	(2&4)[1] 0.07		(2&4)[1] **<0.01**

8. Sniffing ground (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{a}$1.0^{a}	$_{b}$0-1^{b}	1.0^{a}	$_{b}$0-1^{b}	-	0.5
2	$_{a}$0-1^{b}	$_{a}$1.0^{a}	1.0^{a}	$_{a}$1.0^{a}	(3&4)[1] 0.56	1.0
4	$_{a}$1.0	$_{a}$2.5	1.0	$_{a}$1.0	0.12	1.3
P-value between treatments	(C&4) 0.59	-	0.60	(2&4) 0.78		**0.04**

9. Sniffing* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0^{a}	$_{b}$1.0^{a}	$_{b}$1.0^{a}	$_{b}$0-1^{b}	-	0.8
2	1.0	$_{b}$1.0	$_{b}$1.0	$_{a}$1.0	0.76	1.3
4	2.0	$_{a}$3.0	$_{a}$2.0	$_{a}$1.0	0.27	2.3
P-value between treatments	**0.03**	-	(2&4)[1] **0.03**	(2&4)[1] 0.80		**<0.01**

*Sniffing ground + sniffing housing

10. Grooming self (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0^{a}	1.0^{a}	$_{b}$0-1^{b}	$_{a}$1.0^{a}	(1, 2&4)[1] 0.51	0.8
2	1.0^{a}	1.0^{a}	$_{b}$0-1^{b}	$_{b}$0-1^{b}	(1&2)[1] 0.21	0.8
4	2.0^{a}	1.0^{b}	$_{a}$1.0^{b}	$_{b}$0-1^{c}	(1, 2&3)[1] **0.05**	1.5
P-value between treatments	0.14	0.43	-	-		0.38

11. Grooming self*	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	2.5^{a}	1.5^{a}	$_{a}$1.0^{b}	$_{a}$3.0^{a}	(1, 2&4)[1] 0.54	2.3
2	4.0^{a}	1.0^{a}	$_{a}$0-1^{b}	$_{a}$0.5^{b}	(1&2)[1] 0.60	4.3
4	5.5^{a}	2.0^{b}	$_{a}$1.0^{b}	$_{a}$0-1^{c}	(1, 2&3)[1] **0.05**	6.8
P-value between treatments	0.57	0.65	-	-		0.23

*Total number of licks

12. Rubbing head against housing (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}0\text{-}1^{a}$	$_{c}0^{b}$	$_{b}0^{b}$	$_{b}0\text{-}1^{a}$	-	0.1
2	$_{b}0\text{-}1^{b}$	$_{b}0.5^{b}$	$_{a}1.0^{a}$	$_{a}1.0^{a}$	-	0.8
4	$_{a}1.0^{a}$	$_{a}1.0^{a}$	$_{a}1.0^{a}$	$_{a}0\text{-}1^{b}$	(1, 2&3)[1] 0.55	1.3
P-value between treatments	-	-	-	-		**0.03**

13. Rubbing head against Housing (sec h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{b}1.0^{a}$	$_{c}0^{c}$	$_{c}0^{c}$	$_{b}0\text{-}1^{b}$	-	0.5
2	$_{b}1.0^{a}$	$_{b}1.0^{a}$	$_{b}1.0^{a}$	$_{a}2.0^{a}$	-	3.8
4	$_{a}3.5^{a}$	$_{a}6.0^{a}$	$_{a}7.0^{a}$	$_{a}1.0^{b}$	(1, 2&3)[1] 0.27	10.0
P-value between treatments	-	-	-	-		**0.01**

14. Body care* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	2.0	1.0	$_{a}1.0$	1.0	0.60	1.3
2	2.0	1.0	$_{a}1.0$	1.5	0.71	1.9
4	3.5	2.0	$_{a}2.0$	1.0	**0.01**	2.6
P-value between treatments	0.15	0.10	(2&4)[1] 0.10	0.71		0.25

*Grooming self + rubbing head against furniture

15. Housing interaction* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.0^{a}	$_{b}0\text{-}1^{b}$	$_{b}0^{c}$	$_{b}0\text{-}1^{b}$	-	0.3
2	1.0	$_{a}1.0$	$_{a}1.5$	$_{a}1.0$	0.56	1.3
4	3.0	$_{a}1.5$	$_{a}3.0$	$_{a}1.5$	0.07	2.4
P-value between treatments	**0.01**	(2&4)[1] 0.29	(2&4)[1] 0.35	(2&4)[1] 0.59		**<0.01**

*Rubbing head against housing + sniffing housing + licking housing.

16. Licking* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	1.5	1.0	$_{a}$0-1	1.0	0.52	1.0
2	1.0	1.0	$_{a}$0-1	1.0	0.40	0.8
4	3.0	2.0	$_{a}$1.5	0.5	**0.01**	2.4
P-value between treatments	0.14	**0.01**	-	0.67		0.30

*Grooming self + licking housing

17. Stress* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	6.5	5.5	5.5	8.0	0.81	6.6
2 (m)	- 9.0	- 6.0	28.3 25.0	27.6 23.0	(3&4)[1] 0.84 **<0.01**	15.4
4 (m)	- 25.0	32.1 27.0	28.4 25.5	28.1 25.0	(2, 3&4)[1] 0.18 0.57	24.5
P-value between treatments	**<0.01**	**<0.01**	**<0.01** (2&4)[1] 0.97	**<0.01** (2&4)[1] 0.93		**<0.01**

*Leg raising + repositioning + weight shifting + head swinging + butting + vocalising + grooming self; [m]mean values on top row

18. Butting (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{c}$0[a]	$_{b}$0[a]	$_{b}$0[a]	$_{b}$0[a]	-	$_{b}$0
2	$_{b}$0-1[a]	$_{b}$0[b]	$_{a}$0.5[a]	$_{a}$0-1[a]	-	$_{a}$0.3
4	$_{a}$1.0[a]	$_{a}$1.0[a]	$_{a}$0-1[b]	$_{a}$0-1[b]	(1&2)[1] 0.59	$_{a}$0.8
P-value between treatments	-	-	-	-		(2&4)[1] 0.12

19. Frustration* (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	2.5	2.5	2.0	1.5	0.36	2.3
2	2.0	2.0	3.5	3.0	0.49	3.1
4	3.5	7.0	5.5	5.0	**0.01**	5.8
P-value between treatments	0.07	**0.01**	0.20	0.66		0.09

*Head swinging + butting + vocalising

20. Drinking (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{a}1.0^{a}$	$_{b}0^{c}$	$_{a}0\text{-}1^{b}$	$_{a}0\text{-}1^{b}$	-	$_{a}0.5$
2	$_{a}1.0^{a}$	$_{a}1.0^{a}$	$_{a}0\text{-}1^{b}$	$_{b}0^{c}$	-	$_{a}0.5$
4	$_{b}0^{b}$	$_{a}0\text{-}1^{a}$	$_{a}0\text{-}1^{a}$	$_{b}0^{c}$	-	$_{a}0\text{-}1$
P-value between treatments	(C&2)[1] 1.00	-	-	-		-

21. Drinking (sec h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	$_{a}15.5^{a}$	$_{b}0^{c}$	$_{a}6.0^{a}$	$_{a}1.0^{b}$	-	$_{a}10.1$
2	$_{a}4.5^{a}$	$_{a}1.0^{a}$	$_{a}1.0^{a}$	$_{b}0^{b}$	-	$_{a}10.0$
4	$_{b}0^{b}$	$_{a}0\text{-}1^{a}$	$_{b}0\text{-}1^{a}$	$_{b}0^{b}$	-	$_{b}0\text{-}1$
P-value between treatments	(C&2)[1] 0.62	-	-	-		(1&2)[1] 0.64

22. Walking (no. h^{-1})	Hour 1	2	3	4	P-value between hours	Median value for all hours
C	2.0	1.0	2.0	2.0	0.20	1.8
2	2.0	2.0	-	-	1.00	1.0
4	-	-	-	-	-	-
P-value between treatments	0.59	0.44	-	-		**<0.01**

Key:

- Row values with different superscripts are significantly different ($P \leqslant 0.05$).
- Treatment values with different subscripts are significantly different ($P \leqslant 0.05$).
- [1]Numbers/letter in parenthesis represent the treatments/hours to which the following P-value refers.
- Where letters to indicate differences between values are absent, but a significant probability value has been shown, a non-parametric test was performed and therefore no post-hoc test of paired means could be carried out.

- All values are medians unless otherwise stated. Mean values were only used to determine differences between hours.
- The symbol '-' in the treatment/hour box indicates that the cows for that treatment were unable to perform the behaviour in that hour.
- '0-1' denotes the median is zero, but the 1-Sample Sign test indicates that the true median lies between zero and one.
- Bold P-values are used where $P \leq 0.05$.

Repositioning was not observed, and weight shifting was not observed to any major extent for the cows when in treatment C (Table 6.7a2 & 3). When in treatment 2 the cows shifted their weight significantly more frequently during deprivation (Table 6.7a3). There was no significant difference between hours in the frequency of repositioning in treatment 2 or for weight shifting and repositioning in treatment 4.

There was no significant difference in repositioning between treatments 2 and 4 in the last two hours of deprivation (Table 6.7a2). The cows were observed weight shifting significantly more frequently when in treatment 4, compared to treatment 2, in the first, but not the last two hours (Table 6.7a3). Both weight shifting and repositioning significantly increased between treatments, with increasing deprivation time, over the entire four hour period.

Shifting and restlessness type behaviours did not significantly differ between hours for cows when in treatments C and 4 (Table 6.7a4 & 6). Both behaviours significantly increased during deprivation for the cows when in treatment 2. For each hour, and

over the entire deprivation period, both behaviours significantly increased between treatments with increasing deprivation time (Table 6.7a4 & 6).

There were no significant differences between hours for head swinging, body care, licking and frustration-related behaviours for the cows when in treatments C and 2 (Table 6.7a5, 14, 16 & 19). The frequency of body care and licking behaviours significantly decreased with time for the cows when in treatment 4 (Table 6.7a14 & 16). In treatment 4, frustration-related behaviours were increased and there was a tendency for head swinging to be greater in the second hour in comparison to the other hours (Table 6.7a5).

For body care, head swinging, licking and frustration-related behaviours there was no significant difference between treatments for any of the hours, except the second hour when the cows, during treatment 4, significantly increased the frequency of the latter three behaviours, compared to when in treatments C and 2 (Table 6.7a14, 5, 16 & 19, respectively). Over the entire deprivation period there was no significant difference between treatments for head swinging, body care and licking behaviours (Table 6.7a5, 14 & 16). There was a tendency for frustration type behaviours to increase between treatments with increasing deprivation length (P=0.09) (Table 6.7a19).

Nosing ground was not observed, and rubbing head against the housing was rarely observed for the cows when in treatment C (Table 6.7a7 & 12). Whilst undergoing treatment 2 there was a small but significant increase in both behaviours during actual deprivation. The frequency of these behaviours were not clearly affected by time in treatment 4.

The cows nosed the ground and rubbed their heads against the housing significantly more frequently in the first, but not the last two hours, when in treatment 4, compared to treatment 2 (Table 6.7a7 & 12). Both behaviours were increased significantly more for the four than two hour deprivation treatment.

The frequency of sniffing the ground was low for cows in all treatments and was not clearly affected by time or treatment (Table 6.7a8). However, this behaviour increased significantly between treatments with increasing deprivation time, over the entire deprivation period. When in treatment C the cows were observed sniffing significantly less frequently in hour four than one, two or three (Table 6.7a9). There was no significant difference in the frequency of sniffing behaviours performed over time for the cows when in treatments 2 and 4.

In treatment 4, the cows performed sniffing behaviours significantly more frequently in the first, second and third hour compared to when in the other two treatments (Table 6.7a9). In the fourth hour, cows sniffed significantly more frequently when in treatments 2 and 4 compared to treatment C. For the whole of the deprivation period, sniffing behaviours increased significantly between treatments with increasing deprivation length.

The frequency and total number of licks whilst self grooming were significantly lower in the third hour, compared to the other two hours for the cows when in treatment C (Table 6.7a10 & 11). The frequency and total number of licks whilst self grooming declined over time for the cows when in treatment 4, and were significantly lower

during deprivation when in treatment 2. Neither the frequency of bouts nor the number of licks whilst self grooming were clearly affected by treatment (Table 6.7a 10 & 11b).

Rubbing the head against the housing was rarely observed for the cows when in treatment C, and was not clearly affected by time (Table 6.7a13). This behaviour was not significantly different between hours for the cows when in treatment 2. During treatment 4, the cows spent significantly less time in this behaviour in the last hour.

In the first hour, the cows spent significantly more time rubbing their heads against the housing when in treatment 4, compared to the other two treatments (Table 6.7a13). In the fourth hour, this behaviour was performed significantly more whilst undergoing the deprivation treatments, compared to treatment C. For hours two and three, and over the entire deprivation period, this behaviour was increased more for treatment 4 than 2, compared with treatment C.

Housing interaction was rarely observed in treatment C and was not significantly different between hours for the cows when in treatment 2 (Table 6.7a15). There was a tendency for this behaviour to decrease in hours two and four in comparison to hours one and three for the cows when in treatment 4 (P=0.07).

In the first hour, the cows interacted with the housing significantly more frequently when in treatment 4, in comparison to the other two treatments (Table 6.7a15). For hours two to four, cows in treatments 2 and 4 showed increased housing interaction.

Overall there was an increase in housing interaction from treatment C to 2 and from treatment 2 to 4.

There was no significant difference between hours in the performance of stress type behaviours for the cows when in treatments C and 4 (Table 6.7a17). When in treatment 2 the cows increased the frequency of this behaviour during deprivation ($P < 0.01$).

The frequency of stress type behaviours were significantly different between treatments and increased with increasing deprivation length for all hours, and over the entire deprivation period (Table 6.7a17).

Cows were not observed butting when in treatment C (Table 6.7a18). Butting was rarely observed for the cows whilst undergoing treatment 2, and was not clearly affected by time. When in treatment 4, there was a small but significant decrease in butting in the last two hours compared to the first two.

Cows were observed butting most frequently when in treatment 4 for all hours and over the entire deprivation period, and were significantly different from treatment 2 during the first, but not the last two hours or over the entire deprivation period (Table 6.7a18).

The median frequency of drinking in each hour was low for cows during all treatments (Table 6.7a 20). There was no clear effect of treatment on drinking frequency for any of the hours or over the entire deprivation period.

In treatment C the cows spent significantly more time drinking during the first and third hour, in comparison to the other two hours (Table 6.7a21). When in treatments 2 and 4 the time spent drinking was not clearly affected by time.

There was no clear difference between treatments for drinking time during the second and fourth hour (Table 6.7a21). During hours one and three this behaviour decreased for cows when in treatment 4, and there was no difference between treatments C and 2. Over the entire deprivation period, the time spent drinking was significantly lower for treatment 4 in comparison to the other two treatments, which were not significantly different.

Whilst undergoing treatments 2 and 4 the cows were restricted from walking during deprivation (Table 6.7a22). When in treatments C and 2 there was no significant difference between hours in walking activity. No significant difference was observed between treatments C and 2 in the first or second hour for walking frequency (Table 6.7a22). In treatment 2 the cows were observed walking less frequently over the entire deprivation period ($P < 0.01$).

Previous treatment and period had no significant effect on the performance of any behaviour tested ($P>0.10$).

6.4.3.2a Behavioural versus production variable correlations for continuous recordings

In treatment C the cows leg raised more frequently with increasing milk yield ($P=0.01$) (Table 6.8a). There was also a positive correlation between this behaviour

and the number of days to parturition for cows when in treatments C and 2 (r^2=26.2, P=0.03; r^2=34.0, P=0.01, respectively) (Table 6.9a).

Table 6.8a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with milk yield in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Milk yield					
	Correlations (P-value; coefficient)			Regressions (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	**0.01** **0.60**	0.29 0.26	0.43 0.20	-	-	-
Weight shifting	**<0.01** **0.70**	0.17 0.34	0.99 -0.00	0.05 22.6	-	-
Shifting	**0.01** **0.60**	*0.07* *0.44*	0.71 0.09	-	-	-
Head swinging	*0.07* *0.45*	*0.09* *0.41*	*0.09* *0.42*	-	-	-
Restlessness[1]	**0.03** **0.50**	0.16 0.35	0.43 0.20	-	-	-
Stress[1]	**0.04** **0.48**	0.21 0.31	0.39 0.22	-	-	-
Walking	**0.04** **-0.48**	0.58 0.14	-	-	-	-

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section); Bold P-values are used where P≤0.05. [1]Relates to those behaviours that are indicative of this experience.

When in treatment C there was a negative correlation between weight shifting and body condition score (P=0.03) (Table 6.10a), and this behaviour increased with increasing milk yield (r^2=22.6, P=0.05) and the number of days to parturition (r^2=29.3, P=0.02) (Tables 6.8a & 6.9a, respectively).

As milk yield increased the cows performed shifting behaviours more frequently when in treatment C (P=0.01) and there was a tendency for these behaviours to increase when in treatment 2 (P=0.07) (Table 6.8a). Shifting behaviours also

increased the closer the cows were to parturition, when in treatments C and 2 (r^2=24.3, P=0.04; r^2=35.5, P=0.01, respectively) (Table 6.9a).

Table 6.9a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with the number of days to parturition in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Days to parturition					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	**<0.01** **0.66**	**<0.01** **0.66**	0.44 0.19	0.03 26.2	0.01 34.0	-
Weight shifting	**<0.01** **0.77**	0.25 0.28	0.60 0.13	0.02 29.3	-	-
Shifting	**<0.01** **0.68**	**<0.01** **0.61**	0.44 0.19	0.04 24.3	0.01 35.5	-
Restlessness[1]	**0.01** **0.63**	**0.01** **0.64**	0.62 0.13	0.04 24.0	0.01 35.1	-
Stress[1]	**0.01** **0.63**	**0.01** **0.62**	0.78 0.07	0.05 22.1	0.04 23.8	-
Frustration[1]	*0.08* *0.43*	0.25 0.29	0.89 0.05	-	-	-

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05. [1]Relates to those behaviours that are indicative of this experience.

Table 6.10a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with body condition score in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Body condition score					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Weight shifting	**0.03** **-0.50**	0.95 -0.02	0.96 -0.01	-	-	-
Head swinging	0.38 -0.22	0.82 -0.06	**0.05** **-0.47**	-	-	0.04 23.6

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05.

Table 6.11a The significance and coefficient for the average frequency, or duration, of each behaviour recorded over the entire deprivation period, which was significantly correlated with lactation number in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Lactation number					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Sniffing	**0.02** **-0.54**	0.72 0.09	0.78 -0.07		-	-

*Discrepancies between correlation and regression P-values due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$.

There was a negative relationship between head swinging and body condition score for the cows when in treatment 4 (r^2=23.6, P=0.04) (Table 6.10a). The higher yielding cows tended to head swing more frequently when in treatments C, 2 and 4 (P=0.07, P=0.09 & P=0.09, respectively) (Table 6.8a).

In treatment C the higher yielding cows were observed performing restlessness type behaviours more frequently (P=0.03) (Table 6.8a). These behaviours also increased the further into pregnancy the cows were, when in treatments C and 2 (r^2=24.0, P=0.04; r^2=35.1, P=0.01, respectively) (Table 6.9a).

When in treatment C the older cows were observed sniffing less frequently (P=0.02) (Table 6.11a).

In treatment C the frequency of stress-related behaviours and milk yield were positively correlated (P=0.04) (Table 6.8a). There was also a positive relationship between this behaviour and the number of days to parturition for the cows when in treatments C and 2 (r^2=22.1, P=0.05; r^2=23.8, P=0.04, respectively) (Table 6.9a).

In treatment C there was a tendency for those cows closer to parturition to perform frustration type behaviours more frequently (P=0.08) (Table 6.9a), and walk less frequently as milk yield increased (P=0.04) (Table 6.8a).

6.4.3.3a Behavioural correlations for continuous recordings

In treatment C, leg raising was significantly positively correlated with weight shifting, head swinging and frustration-related behaviours (Table 6.12a). When in treatment 2 the cows significantly increased leg raising behaviour with increasing head swinging, frustration type behaviours and housing interaction (Table 6.12a). There was a positive relationship between leg raising and frustration-related behaviours for the cows when in treatment 4 (r^2=30.4, P=0.01) (Table 6.12a).

Table 6.12a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with leg raising, in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Leg raising					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Weight shifting	**<0.01** **0.67**	0.97 0.01	0.21 0.31	<0.01 86.1	-	-
Head swinging	**<0.01** **0.92**	**<0.01** **0.79**	0.11 0.39	<0.01 86.4	<0.01 47.8	-
Housing interaction	0.42 0.21	**0.01** **0.59**	0.54 0.15	-	0.03 21.8	-
Frustration[1]	**<0.01** **0.88**	**<0.01** **0.65**	**0.03** **0.51**	<0.01 77.9	0.01 28.7	0.01 30.4

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05. [1]Relates to those behaviours that are indicative of this experience.

When in treatment 2 the cows head swung significantly more often the more they shifted, sniffed, licked, interacted with the housing and performed body care

behaviours (Table 6.13a). Head swinging also increased with increasing shifting behaviours for the cows when in treatment C (r^2=85.6, P < 0.01) (Table 6.13a).

Table 6.13a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with head swinging, in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Head swinging					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Shifting	**<0.01** **0.91**	**0.03** **0.50**	0.36 0.23	<0.01 85.6	0.02 23.5	-
Sniffing	0.62 0.13	**0.01** **0.58**	0.46 0.19	-	0.01 30.9	-
Body care	0.30 0.26	**<0.01** **0.67**	0.23 0.30	-	<0.01 43.5	-
Housing interaction	0.76 0.08	**<0.01** **0.76**	0.11 0.39	-	<0.01 51.1	-
Licking	0.15 0.35	**<0.01** **0.65**	0.27 0.28	-	<0.01 41.9	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05.

Nosing the ground was performed significantly more frequently as the cows sniffed more, rubbed their heads against the housing more and interacted with the housing more during treatment 4 (Table 6.14a). When in treatment 2 there was a trend towards a positive correlation between nosing the ground and repositioning (P=0.08), and between nosing the ground and the total number of licks whilst self grooming in treatment 4 (P=0.06) (Table 6.14a).

In treatment 4 the cows sniffed the ground significantly more frequently the more they leg raised, nosed the ground, interacted with the housing and the more restless- and stress-related behaviours they exhibited (Table 6.15a). Ground sniffing was also

significantly positively correlated with the time spent rubbing the head against the housing, housing interaction and frustration-related behaviours for the cows when in treatment 2 (Table 6.15a).

Table 6.14a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with nosing ground in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Nosing ground					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Repositioning	N/A	*0.08* *0.43*	0.38 0.22	-	-	-
Sniffing	N/A	0.60 0.13	**0.04** **0.49**	-	-	0.05 17.1
Total number of licks whilst grooming self	N/A	0.64 -0.12	*0.06* *0.45*	-	-	-
Rubbing head against housing	N/A	0.50 -0.17	**0.01** **0.61**	-	-	0.01 30.0
Time spent rubbing head against housing	N/A	0.41 -0.21	**<0.01** **0.62**	-	-	-
Housing interaction	N/A	0.28 0.27	**<0.01** **0.75**	-	-	0.01 28.5

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$.

Sniffing behaviours increased with increasing body care behaviours for the cows during treatment C (r^2=39.9, $P < 0.01$) (Table 6.16a). In treatment 2, leg raising, repositioning, shifting, the time spent rubbing the head against the housing, body care, restlessness- and stress-related behaviours were all performed significantly more often as sniffing behaviours increased (Table 6.16a). Leg raising, shifting, restlessness- and

stress-related behaviours were also significantly positively correlated with sniffing behaviours for the cows when in treatment 4 (Table 6.16a).

Table 6.15a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with sniffing the ground, in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Sniffing ground					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	0.85 0.05	0.15 0.35	**0.02** **0.56**	-	-	-
Nose ground	N/A	0.74 -0.08	**0.04** **0.47**	-	-	0.04 18.2
Restlessness[1]	0.91 0.03	0.12 0.39	**0.02** **0.54**	-	-	-
Time spent rubbing head against housing	N/A	**<0.01** **0.66**	0.41 -0.21	-	**<0.01** 63.0	-
Housing interaction	0.18 0.33	**0.01** **0.57**	**0.02** **0.56**	-	0.02 26.3	-
Stress[1]	0.61 0.13	0.11 0.39	**0.01** **0.58**	-	-	-
Frustration[1]	0.73 0.09	**0.05** **0.47**	0.22 0.31	-	-	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$. [1]Relates to those behaviours that are indicative of this experience.

In treatment 2 the more restless- and stress-related behaviours the cows exhibited, the more frequently they interacted with the housing (r^2=20.8, P=0.03; r^2=26.5, P=0.02, respectively) (Table 6.17a).

Table 6.16a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with sniffing behaviours in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Sniffing behaviours					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Leg raising	0.59 0.14	**0.01** **0.59**	**0.01** **0.59**	-	**<0.01** **46.6**	-
Repositioning	N/A	**0.02** **0.54**	0.10 0.40	-	**0.03** **20.4**	-
Shifting	0.68 0.11	**0.03** **0.50**	**0.02** **0.53**	-	**<0.01** **40.8**	-
Restlessness[1]	0.68 0.10	**0.02** **0.56**	**0.01** **0.60**	-	**<0.01** **46.5**	-
Time spent rubbing head against housing	N/A	**<0.01** **0.61**	0.43 0.20	-	**<0.01** **41.8**	-
Body care	**<0.01** **0.60**	**0.04** **0.49**	0.22 0.30	**<0.01** **39.9**	-	-
Stress[1]	0.36 0.23	**0.01** **0.57**	**0.01** **0.60**	-	**<0.01** **45.0**	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$. [1]Relates to those behaviours that are indicative of this experience.

Table 6.17a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with housing interaction in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Housing interaction					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Restlessness[1]	0.63 0.12	**0.05** **0.48**	0.25 0.28	-	**0.03** **20.8**	-
Stress[1]	0.24 0.29	**0.04** **0.50**	0.14 0.36	-	**0.02** **26.5**	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where $P \leq 0.05$. [1]Relates to those behaviours that are indicative of this experience.

Sniffing behaviours, self grooming, body care behaviours, housing interaction and licking behaviours all significantly increased as the cows exhibited more frustration-related behaviours in treatment 2 (Table 6.18a). When in treatment 4 the cows interacted with the housing more frequently the more they performed frustration-related behaviours (P=0.05) (Table 6.18a). There was a positive relationship between walking and repositioning for the cows when in treatment 2 (r^2=31.8, P=0.01) (Table 6.19a).

Table 6.18a The significance and coefficient for the average frequency of each behaviour, recorded over the entire deprivation period, which was significantly correlated with frustration type behaviours in at least one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Frustration[1]					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Sniffing	0.42 0.20	**<0.01** **0.67**	0.24 0.30	-	0.02 27.5	-
Grooming self	0.15 0.36	**0.02** **0.55**	0.30 0.26	-	<0.01 52.1	-
Body care	0.17 0.33	**<0.01** **0.73**	0.10 0.40	-	<0.01 57.8	-
Housing interaction	0.62 0.13	**<0.01** **0.82**	**0.05** **0.47**	-	<0.01 48.0	-
Licking behaviours	0.11 0.39	**<0.01** **0.68**	0.13 0.37	-	<0.01 54.9	-

*Discrepancies between correlation and regression P-values are due to ranking of data for correlations (see statistical analysis section). Bold P-values are used where P≤0.05. [1]Relates to those behaviours that are indicative of this experience.

Table 6.19a The significance and coefficient for the average frequency, or duration, of each behaviour, recorded over the entire deprivation period, which was significantly correlated with walking, in one of the control (C), 2 h (2) or 4 h (4) treatments, and the r^2 value for significant (P≤0.05) regressions

	Walking					
	Correlations (P-value; coefficient)			Regressions* (P-value; r^2)		
Behaviour	C	2	4	C	2	4
Repositioning	N/A	**0.02** **0.56**	N/A	-	0.01 31.8	-

*Discrepancy between correlation and regression P-value is due to ranking of data for correlations (see statistical analysis section). Bold P-value is used where P≤0.05

6.4.3.4a Instantaneous recordings

Each sub-table (1 to 8) in Table 6.20a shows the differences between values for each hour (by row) and for each treatment (by column), for a specific behaviour.

When the cows were in treatments C and 2 there was no significant difference between hours in the amount of time spent feeding, lying, lying ruminating, in all standing behaviours (standing and standing ruminating), in all lying behaviours (lying and lying ruminating) and in all standing behaviours including feeding (Table 6.20a1, 3, 5, 6, 7 & 8, respectively). For all of these behaviours there was no significant treatment effect during the first two hours of deprivation. Over the entire period, when in treatment 2 the cows spent significantly less time feeding, lying, lying ruminating and in all lying behaviours, but more time in all standing behaviours and in all standing behaviours including feeding, in comparison to when in treatment C (Table 6.20a1, 3, 5, 7, 6 & 8, respectively).

Table 6.20a The average time (min h^{-1}) that each behaviour was performed per hour, and for all four hours, during the deprivation period, for all of the experimental cows in the control (C), 2 h (2) and 4 h (4) treatments

1. Feeding	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours (min h^{-1})
C[m]	- 20.0	15.8 15.0	10.8 10.0	15.3 15.0	(2,3&4)[1] 4.71	(2,3&4)[1] 0.24 0.29	59.2
2	17.5	12.5	-	-	-	0.80	32.8
4	-	-	-	-	-	-	-
SED ±	-	-	-	-			4.60
P-value between treatments	0.62	0.64	-	-			**<0.01**

[m]Mean values on top row; [1]Numbers in parenthesis represent the hours to which the following P-value/SED refers

2. Standing	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours (min h^{-1})
C[(m)¶]	10.8 10.0	- 5.0	- 12.5	13.6 10.0	(1&4)[1] 3.57	(1&4)[1] 0.39 0.75	45.8
2[(m)]	15.8 -	11.7 12.5	26.1 25.0	28.3 30.0	3.48	**<0.01**	81.9
4[(m)]	38.1 -	35.6 35.0	26.7 25.0	36.4 37.5	3.26	**<0.01**	136.7
SED ±	3.43	(2&4)[1] 2.72	(2&4)[1] 3.68	3.63			8.69
P-value between treatments	**<0.01**	**<0.01** (2&4)[1] **<0.01**	**<0.01** (2&4)[1] 0.90	**<0.01** (2&4)[1] **0.04**			**<0.01**

[m]Mean values on top row; [¶]Used back transformed data to calculate mean values; [1]Numbers in parenthesis represent the hours/treatments to which the following P-value/SED refers

3. Lying	Hour 1	2	3	4	P-value between hours	Mean time for all hours[¶] (min h^{-1})
C	$_{a}$2.5	7.5	7.5	2.5	0.29	26.4
2	$_{a}$0.0[a]	2.5[a]	-	-	-	14.2
4	-	-	-	-	-	-
SED ±	-	-	-	-		7.00
P-value between treatments	-	0.25	-	-		**0.01**

[¶]Used back transformed data to calculate mean values

4. Standing ruminating	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours[¶] (min h^{-1})
C	2.5[a]	$_{c}$0.0[b]	5.0[a]	$_{c}$0.0[b]	-	(1&3)[1] 0.21	26.9
2[(m)]	- 5.0	- $_{b}$7.5	33.9 35.0	31.7 $_{a}$30.0	(3&4)[1] 3.84	(3&4)[1] 0.47 **<0.01**	75.7
4[(m)]	21.9 22.5	24.4 $_{a}$25.0	33.3 35.0	23.6 $_{b}$22.5	3.26	**<0.01**	100.2
SED ±	-	-	(2&4)[1] 3.68	(2&4)[1] 3.41			10.78
P-value between treatments	**<0.01**	(2&4)[1] **0.03**	**<0.01** (2&4)[1] 0.90	(2&4)[1] **<0.01**			**<0.01**

[m]Mean values on top row; [1]Numbers in parenthesis represent the hours/ treatments to which the following P-value/SED refers;
[¶]Used back transformed data to calculate mean values

5. Lying ruminating	Hour 1	2	3	4	P-value between hours	Mean time for all hours (min h^{-1})
C	7.5	7.5	22.5	5.0	0.77	65.6
2	12.5	5.0	-	-	0.72	31.4
4	-	-	-	-	-	-
SED ±	-	-	-	-		12.06
P-value between treatments	0.80	0.56	-	-		**<0.01**

6. All standing behaviours*	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours (min h^{-1})
C	19.2 17.5	- 7.5	21.9 20.0	- 22.5	-	(1&3)[1] 0.24 0.32	81.9
2[(m)]	20.8 -	22.8 20.0	-	-	5.32	0.59	163.6
4	-	-	-	-	-	-	-
SED ±	-	-	-	-			15.99
P-value between treatments	0.73	0.08	-	-			**<0.01**

[m]Mean values on top row; *standing + ruminating standing; [1]Numbers in parenthesis represent the hours to which the following P-value/SED refers

7. All lying behaviours*	Hour 1	2	3	4	SED ±	P-value between hours	Median time for all hours (min h^{-1})
C[(m)¶]	17.2 22.5	- 32.5	21.4 32.5	13.7 12.5	(1,3&4)[1] 6.51	(1,3&4)[1] 0.43 0.42	100.0
2[(m)]	22.5 22.5	20.0	-	-	-	0.78	55.0
4	-	-	-	-	-	-	-
SED ±	6.69	-	-	-			-
P-value between treatments	0.85	0.59	-	-			**0.01**

[m]Mean values; *lying + ruminating lying; [1]Numbers in parenthesis represent the hours to which the following P-value/SED refers; [¶]Used back transformed data to calculate mean values

8. All standing behaviours & Feeding*	Hour 1	2	3	4	SED ±	P-value between hours	Mean time for all hours[1] (min h^{-1})
C[(m)]	36.4 37.5	- 27.5	32.8 27.5	- 47.5	(1&3)[1] 6.20	(1&3)[1] 0.30 0.42	128.5
2[(m)]	37.5 37.5	- 40.0	-	-	-	0.78	194.1
4	-	-	-	-	-	-	-
SED ±	5.52	-	-	-			15.64
P-value between treatments	0.85	0.59	-	-			**<0.01**

[m]Mean values; *standing + ruminating standing + feeding; [1]Numbers in parenthesis represent the hours to which the following P-value/SED refers

Key:

- Row values with different superscripts are significantly different (P≤0.05).
- Treatment values with different subscripts are significantly different (P≤0.05).
- [1]Numbers in parenthesis represent the treatments/hours to which the following P-value refers.
- Where letters to indicate differences between values are absent, but a significant probability value has been shown, a non-parametric test was performed and therefore no post-hoc test of paired means could be carried out.
- All values are medians unless otherwise stated.
- The symbol '-' in the treatment/hour box indicates that the cows for that treatment were unable to perform the behaviour in that hour, or this was the only behaviour that they were able to perform.
- Bold P-values are used where P≤0.05.

Standing time did not change over time for the cows when in treatment C (P=0.75) (Table 6.20a2). In this treatment, the cows were only observed standing ruminating in the first and third hour of the deprivation period, which were not significantly

different (Table 6.20a4). In treatment 2 the cows spent significantly longer standing and standing ruminating during deprivation (hours 3 & 4) in comparison to the first two hours (Table 6.20a2 & 4). When in treatment 4 the cows spent the most time standing in the first hour and the least time in the third hour, and the least time standing ruminating in the first hour and the most time in this activity during the third hour.

The time spent standing and standing ruminating significantly increased with increasing deprivation time in the first and second hour and over the entire deprivation period (Table 6.20a 2 & 4). In the third hour, the cows spent significantly longer performing these behaviours when in treatments 2 and 4, which were not significantly different, compared to treatment C. The cows spent longer standing when in the deprivation treatments compared to treatment C, in the fourth hour ($P < 0.01$) (Table 6.20a2). In the same hour the time spent standing ruminating was greatest when the cows were in treatment 2 and least in treatment C (Table 6.20a4).

When in treatment C the cows spent most of their time lying ruminating (29.3%), then feeding (26.4%), standing (20.5%), standing ruminating (12.0%) and the least time lying (11.8%), over the entire deprivation period. In contrast, in treatment 2 the cows spent most time standing (34.7%), then standing ruminating (32.1%), approximately equal amounts of time lying ruminating and feeding (13.3 and 13.9%, respectively) and the least time lying (6.0%). In treatment 4 the cows spent 57.7% of their time standing and 42.3% of their time standing ruminating.

Previous treatment had no significant effect on the performance of any behaviour tested (P>0.10).

6.4.3.5a Behavioural versus production variable correlations for instantaneous recordings

When in treatment C the cows spent more time standing ruminating and less time lying ruminating as milk yield increased (r^2=42.0, P<0.01; r^2=25.2, P=0.02, respectively) (Table 6.21a). There was a significant positive relationship between milk yield and all standing behaviours and all standing behaviours including feeding for the cows when in treatments C and 2 (Table 6.21a). In both of these treatments the higher yielding cows spent significantly less time in all lying behaviours (Table 6.21a).

When in treatment C, body condition score was significantly positively correlated with lying ruminating and all lying behaviours, but significantly negatively correlated with standing ruminating, all standing behaviours and all standing including feeding behaviours (Table 6.21a).

In treatment C, as the cows came closer to parturition they spent longer standing ruminating and tended to spend less time lying ruminating (r^2=32.5, P=0.01; P=0.06, respectively) (Table 6.21a).

In treatment 2 there was a tendency for lactation number to be negatively correlated with the time spent in all standing behaviours (P=0.06) (Table 6.21a). There was a

significant positive relationship between lying ruminating and lactation number for the cows when in treatments C and 2 (Table 6.21a).

Table 6.21a The significance and coefficient for the average duration of each behaviour, recorded over the entire deprivation period, which was significantly correlated with milk yield, body condition score, the number of days to parturition, and lactation number in at least one of the control (C), 2 h (2) and 4 h (4) treatments, and the r^2 value for significant ($P \leq 0.05$) regressions

	Correlation (P-value; coefficient)			r^2-value		
	Milk yield					
Behaviour	C	2	4	C	2	4
Standing ruminating	**<0.01** **0.67**	0.13 0.37	0.40 0.21	<0.01 42.0	-	-
Lying ruminating	**0.02** **-0.54**	0.19 -0.32	N/A	0.02 25.2	-	-
All standing*	**0.02** **0.54**	**0.01** **0.57**	N/A	0.02 25.1	0.01 27.9	-
All lying	**0.04** **-0.50**	**0.04** **-0.49**	N/A	0.04 20.1	0.04 18.7	-
All standing and feeding	**0.04** **0.50**	**0.04** **0.49**	N/A	0.04 20.1	0.04 18.7	-
	Body condition score					
Standing ruminating	**0.02** **-0.55**	0.33 -0.24	0.45 -0.19	0.02 26.3	-	-
Lying ruminating	**0.03** **0.51**	0.30 0.26	N/A	0.03 21.1	-	-
All standing*	**0.05** **-0.47**	0.18 -0.33	N/A	0.05 17.7	-	-
All lying	**0.03** **0.50**	0.15 0.36	N/A	0.03 20.6	-	-
All standing and feeding	**0.03** **-0.50**	0.15 -0.36	N/A	0.03 20.6	-	-
	Days to parturition					
Standing ruminating	**0.01** **0.60**	0.10 0.40	0.91 0.03	0.01 32.5	-	-
Lying ruminating	*0.06* *-0.46*	0.31 -0.25	N/A	-	-	-
	Lactation number					
All standing*	0.27 -0.27	*0.06* *-0.45*	N/A	-	-	-
Lying ruminating	**0.04** **0.48**	**0.03** **0.50**	N/A	0.04 18.5	0.03 20.9	-

Bold P-values are used where $P \leq 0.05$; Italicised P-values are used where $P < 0.10$. *Does not include feeding.

6.4.3.6a Behavioural correlations for instantaneous recordings

In treatment 2 feeding was negatively correlated with standing (r^2=33.6, P=0.01) and positively correlated with ruminating standing (r^2=16.3, P=0.05).

6.4.4a Lying position and changes in posture post-deprivation

No changes in posture were observed during the first 45 min post-deprivation for any of the experimental cows observed lying in any treatment. All of the experimental cows, for each treatment, observed lying during this period preferred to adopt a lateral lying position. There appeared to be a switch to left side laterality in the deprived cows when lying, but this was not possible to test statistically (Table 6.22a).

There was a tendency for the total number of cows observed lying within the first 45 min post-deprivation and the length of deprivation to be related (P=0.10). When in treatment C, 75% of the cows chose to lie within the first 45 min following the deprivation period, compared with 42 and 33% when in treatments 2 and 4, respectively (Table 6.22a).

Table 6.22a The number of experimental cows that were observed lying on their left or right side during the first hour post-deprivation, for the control (C), 2 h (2) and 4 h (4) treatments

Lying side	C	2	4
Left	2	4	3
Right	7	1	1
Total	9 (75%)	5 (42%)	4 (33%)

When in treatments C, 2 and 4, those cows not observed lying during this period were primarily feeding, which tended to increase with deprivation time (30, 35 & 38 min, respectively. P=0.08). However, only three of the cows were observed feeding when in treatment C, thus providing a small sample size for this calculation.

6.4.5a Post-deprivation video observations

Within the first eight hours post-deprivation (14.20–22.15 h) the cows spent significantly longer feeding (30.8 and 27.8 min, respectively) when in treatments 2 and 4, compared to treatment C (Table 6.23a), with there being no significant difference between treatments 2 and 4. From Fig. 6.2a, it appeared that the greatest increase in feeding time between the cows when in treatment 2, compared to treatment C, took place in the first and third hour (17.20 and 19.20 h), post-milking. In treatment 4 the cows, in addition to these two hours, also greatly increased their feeding time in the second and fourth hour (18.20 and 20.20 h), post-milking, compared to treatment C (Fig. 6.2a). However, when in treatments 2 and 4 the cows spent longer feeding in each hour of the first eight hour period, in comparison to treatment C, except for the second hour post-milking when in treatment 2 (Fig. 6.2a).

There was no significant difference in feeding time between treatments in the second, third or final eight hour period (Table 6.23a). In the fourth eight hour period (hours 25–32, post-deprivation) the cows spent significantly longer feeding in treatment 2, compared to the other two treatments, which were not significantly different (Table 6.23a). During this period, when in treatment 2, the cows spent 16.4 and 17.5 min longer feeding, compared to treatments C and 4, respectively. This increase in feeding time primarily took place during the 28th (17.20 h), 30th (19.20 h) and 31st (20.20h)

hours, post-deprivation (Fig. 6.2a). Over the entire 41 h period, when in treatment 2 the cows spent the greatest amount of time feeding and in treatment C the least time (Table 6.23a, hours 1 to 41). There was only a significant difference in feeding time between these two treatments, during this period. The proportion of time spent feeding over the 41 h period when the cows were in treatments C, 2 and 4 was 11.9, 14.7 and 13.7%, respectively. When the data for the deprivation period was included the cows spent the longest time feeding in treatment 2, and the least in treatment 4 (Table 6.23a, hours – 4 to 41). The SED suggests that there was only a significant difference between these two treatments, during this period.

Table 6.23a The mean time (min) spent feeding every eight hours, for the first 41 hours immediately following lying deprivation, for the whole period (hours 1-41) and for the whole period including the deprivation period (hours-4-41), for all the experimental animals in the control (C), 2 h (2) and 4 h (4) treatments

Hour, post-deprivation. (actual time)	Treatment			SED ±	P-value
	C	2	4		
1 – 8¶ (1420-2215)	70.1 (1.846)	100.9 (2.004)	97.9 (1.991)	(0.0510)	**0.01**
9-16* (2220-0615)	0[a]	2.5[a]	0[a]	-	-
17-24 (0620-1415)	94.4	110.0	98.1	8.45	0.17
25-32 (1420-2215)	64.2	80.6	63.1	7.24	**0.04**
33-41 (2220-0655)	15.28	14.2	13.3	3.89	0.88
1-41 (1420-0655)	254.4	315.3	287.8	19.02	**0.01**
-4-41 (1020-0655)	313.6	348.1	287.8	19.6	**0.02**

¶ Used back transformed data to calculate values. Transformed values in parenthesis. Bold P-values are used where P≤0.05. Values with different superscripts are significantly different (P≤0.05). *Median values

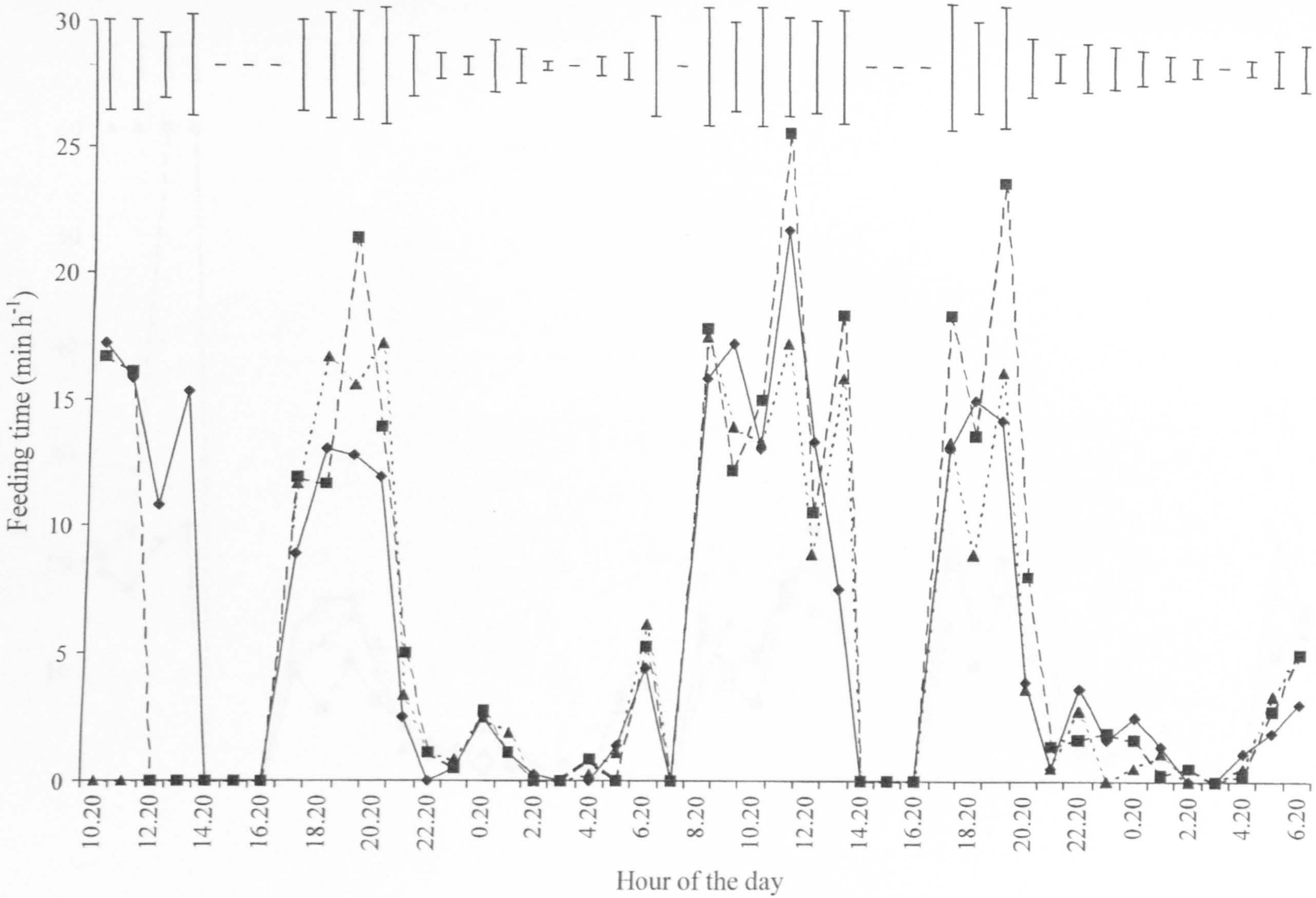


Fig 6.2a The mean time (min h^{-1}) spent feeding for all the experimental animals in the control ——◆—— , 2 h deprivation ----■---- , and 4 h deprivation ……▲…… treatments (I = 1 SED)

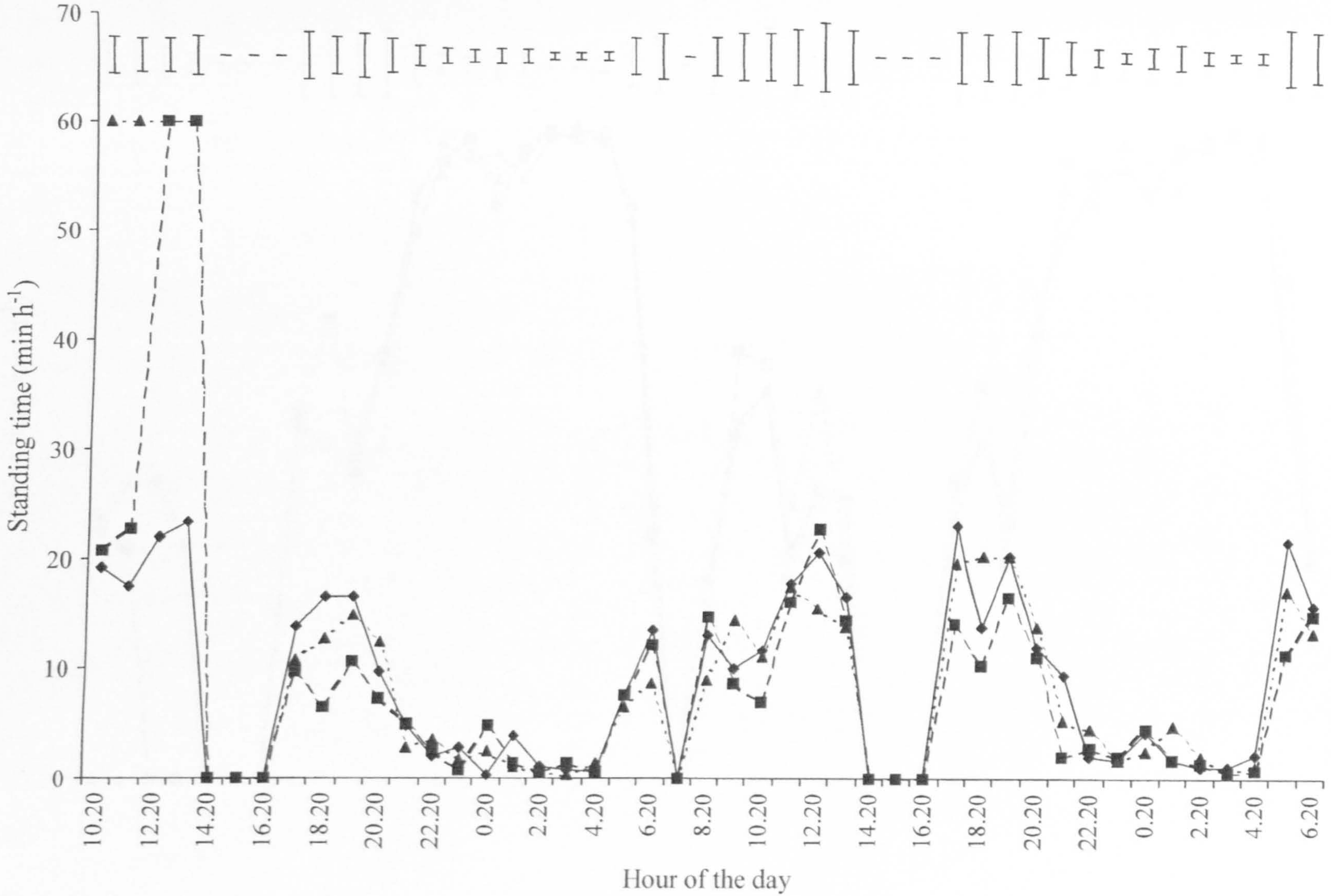


Fig 6.3a The mean time (min h^{-1}) spent standing only for all the experimental animals in the control ——◆——, 2 h deprivation ----■----, and 4 h deprivation ·····▲····· treatments (I = 1 SED)

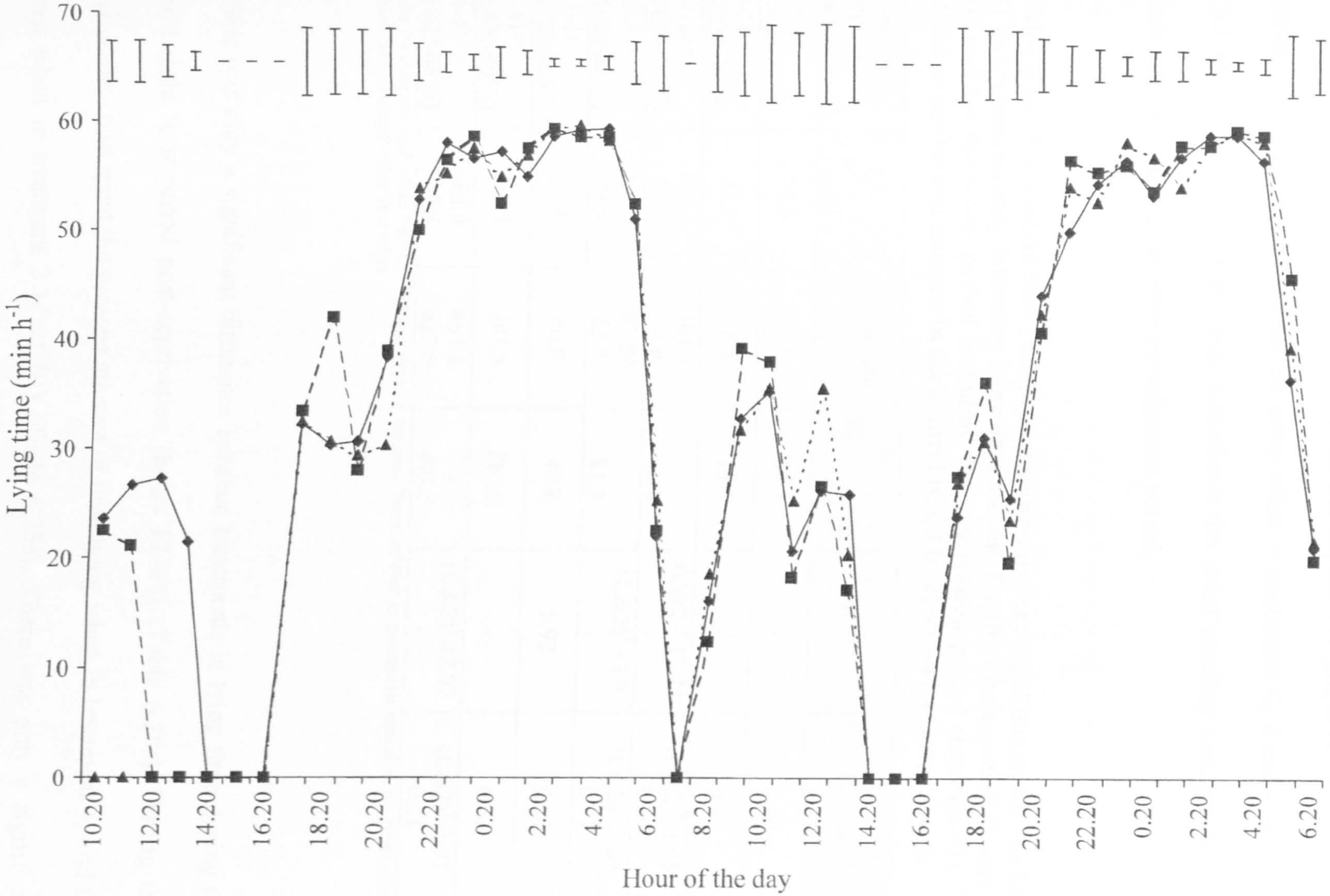


Fig 6.4a The mean time (min h^{-1}) spent lying only for all the experimental animals in the control ——◆—— , 2 h deprivation ----■---- , and 4 h deprivation ·····▲····· treatments (I = 1 SED)

There was no significant difference between treatments in the amount of time spent standing for any of the eight hour periods, over the 41 h period and over this period when the deprivation period was included (Table 6.24a). The proportion of time spent standing over the 41 h period for the cows when in treatments C, 2 and 4 was 15.9, 13.1 and 12.5%, respectively. Fig. 6.3a shows the mean standing time per hour for each treatment during the entire experimental period.

Table 6.24a The median time (min) spent standing every eight hours, for the first 41 hours immediately following lying deprivation, for the whole period (hours 1-41) and for the whole period including the deprivation period (hours-4-41), for all the experimental animals in the control (C), 2 h (2) and 4 h (4) treatments

Hour, post-deprivation. (actual time)	Treatment C	2	4	SED ±	P-value
1 – 8 (1420-2215)	73.3	51.5	47.5	-	0.16
9-16 (2220-0615)	17.5	16.8	13.3	-	0.75
17-24[m] (0620-1415)	109.7 65.0	101.7 74.0	- 60.8	(C&2)[1] 17.73 -	(C&2)[1] 0.66 0.60
25-32[m] (1420-2215)	109.4 99.5	83.89 85.5	- 81.3	(C&2)[1] 13.9 -	(C&2)[1] 0.09 0.57
33-41 (2220-0655)	51.4	40.0	47.8	5.92	0.16
1-41[m] (1420-0655)	337.5	281.8	263.0	-	0.12
-4-41[m] (1020-0655)	465.0 387.5	463.1 422.5	- 492.5	(C&2)[1] 47.07 -	(C&2)[1] 0.97 0.21

Bold P-values are used where P≤0.05. [m]Mean values on top row. [1]Number/letter in parenthesis represents the treatments to which the following P-value/SED refers

There was only a significant difference between treatments in lying time during the third eight hour period post-deprivation (hours 17–24) (Table 6.25a). During this period the cows spent the greatest amount of time lying when in treatment 4, and the least when in treatment 2 ($P < 0.01$) (Table 6.25a). There was only a significant difference in lying times between these two treatments, during this period. It would

appear that when in treatment 4 the cows spent the greatest amount of time lying in hours 22 and 23 post deprivation (11.20 and 12.20 h), in comparison to the other two treatments (Fig. 6.4a). There was no treatment effect on lying time over the 41 h period (P=0.92), or over this time when the deprivation period was included (P=0.28) (Table 6.25a). The proportion of time spent lying over the 41 h period for the cows when in treatments C, 2 and 4 was 72.2, 72.2 and 73.8%, respectively. Previous treatment had no significant effect on the performance of any post-deprivation behaviour tested (P>0.10).

Table 6.25a The mean time (min) spent lying every eight hours, for the first 41 hours immediately following lying deprivation, for the whole period (hours 1-41) and for the whole period including the deprivation period (hours-4-41), for all the experimental animals in the control (C), 2 h (2) and 4 h (4) treatments

Hour, post-deprivation. (actual time)	Treatment C	2	4	SED ±	P-value
1 – 8[¶c] (1420-2215)	235.5 (5.5)	239.5 (5.7)	231.2 (5.3)	(0.77)	0.88
9-16[*] (2220-0615)	455.3 452.5	454.2 462.5	- 465.0	(C&2)[1] 6.48 -	(C&2)[1] 0.87 0.70
17-24 (0620-1415)	184.7	177.2	195.6	18.31	**<0.01**
25-32 (1420-2215)	202.5	211.7	208.1	16.89	0.86
33-41[*] (2220-0655)	- 457.5	465.8 465.0	458.9 475.0	(2&4)[1] 9.04 -	(2&4)[1] 0.54 0.16
1-41[¶c] (1420-0655)	1537.2 (236.3)	1550.8 (240.5)	1557.4 (242.6)	(15.50)	0.92
-4-41[¶c] (1020-0655)	1642.9 (269.9)	1595.9 (254.7)	1557.4 (242.6)	(17.02)	0.28

[¶] Used back transformed data to calculate values. Transformed values in parentheses. [c]Transformed values in parentheses are expressed as min^2, number x 10^4. Bold P-values are used where P≤0.05. *Median values on bottom row. [1]Number/letter in parenthesis represents the treatments to which the following P-value/SED refers

In treatment C the cows maintained a greater cumulative feeding time compared to when in treatments 2 and 4 for the first 23 hours, post-deprivation (Fig. 6.5a). The treatment 2 feeding time per hour drew closer to those recorded for treatment C,

during this period. Between hours 24 to 27 inclusive the feeding time of the cows when in these two treatments were approximately the same. In the 28th hour the feeding time of the cows when in treatment 2 exceeded that of treatment C. This increase continued and peaked in the 30th hour post-deprivation, and was maintained for the remaining 11 hours. Over the 41 h period, in treatment 4 the cows spent the least time feeding, and never achieved a similar accumulated feeding time compared to when in treatments C and 2. The feeding time of the cows when in treatment 4 were closest to those recorded in treatment 2 between hours 19 to 22, post deprivation. However, this appeared to be only a short-term compensation measure, as this difference increased again slightly after the 23rd hour to the end of the observation period.

The cows, when in treatment C had the greatest, and in treatment 4 the lowest, accumulated lying time per hour, over the 41 h period (Fig. 6.6a). The cows were unable to increase their lying time in treatments 4 and 2 to match those recorded when in treatments 2 and C, respectively. Therefore, over the 41 h period the accumulated lying times for each of the three treatments followed approximately the same pattern of increase, and remained at approximately the same time apart.

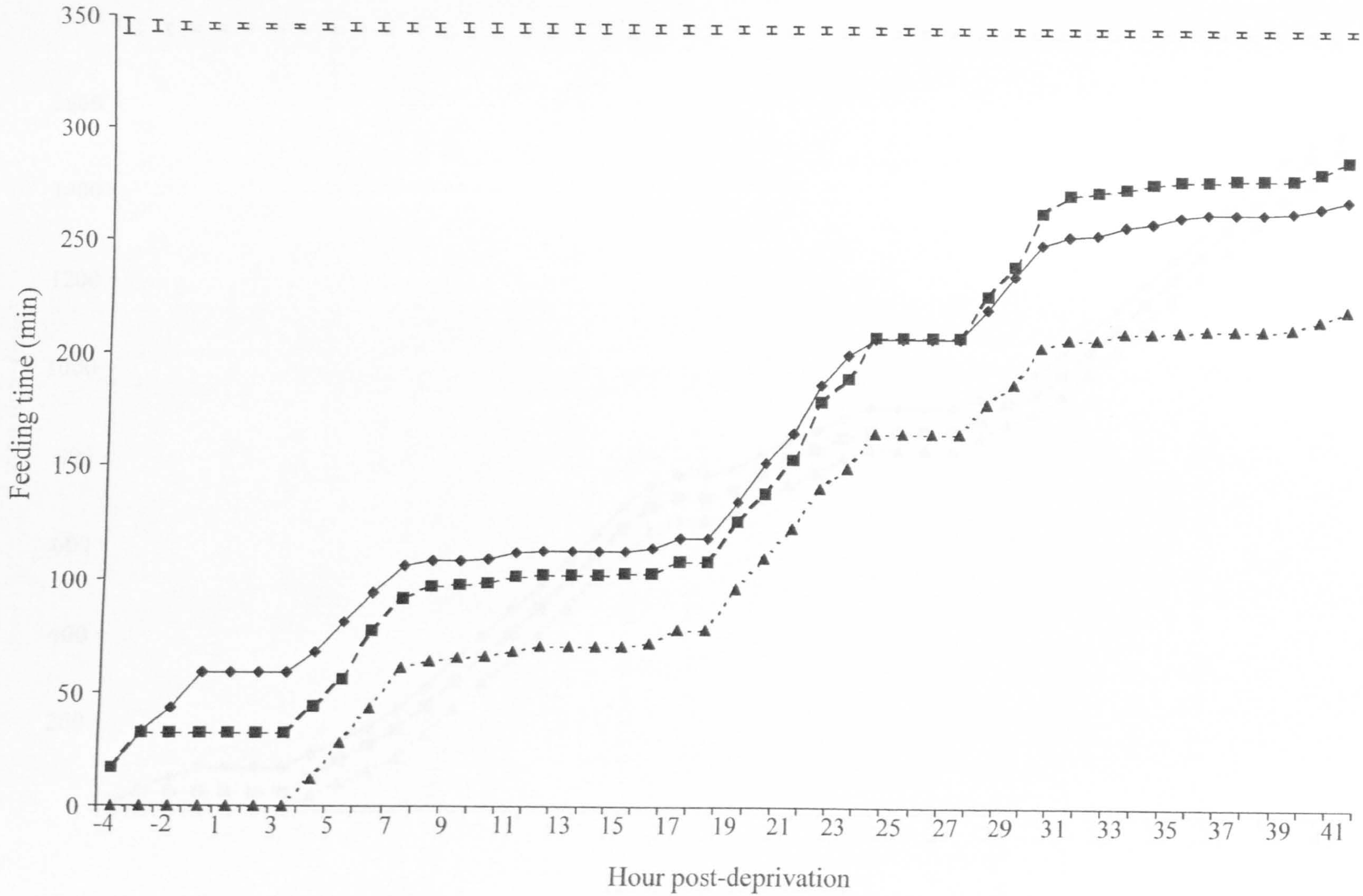


Fig 6.5a The accumulated feeding time (min) per hour for all the experimental animals in the control ——◆—— , 2 h deprivation ---■---- , and 4 h deprivation ·····▲····· treatments post deprivation (hours 1 to 40), and for the four hour deprivation period (hours –4 to –1) (I = 1 SED)

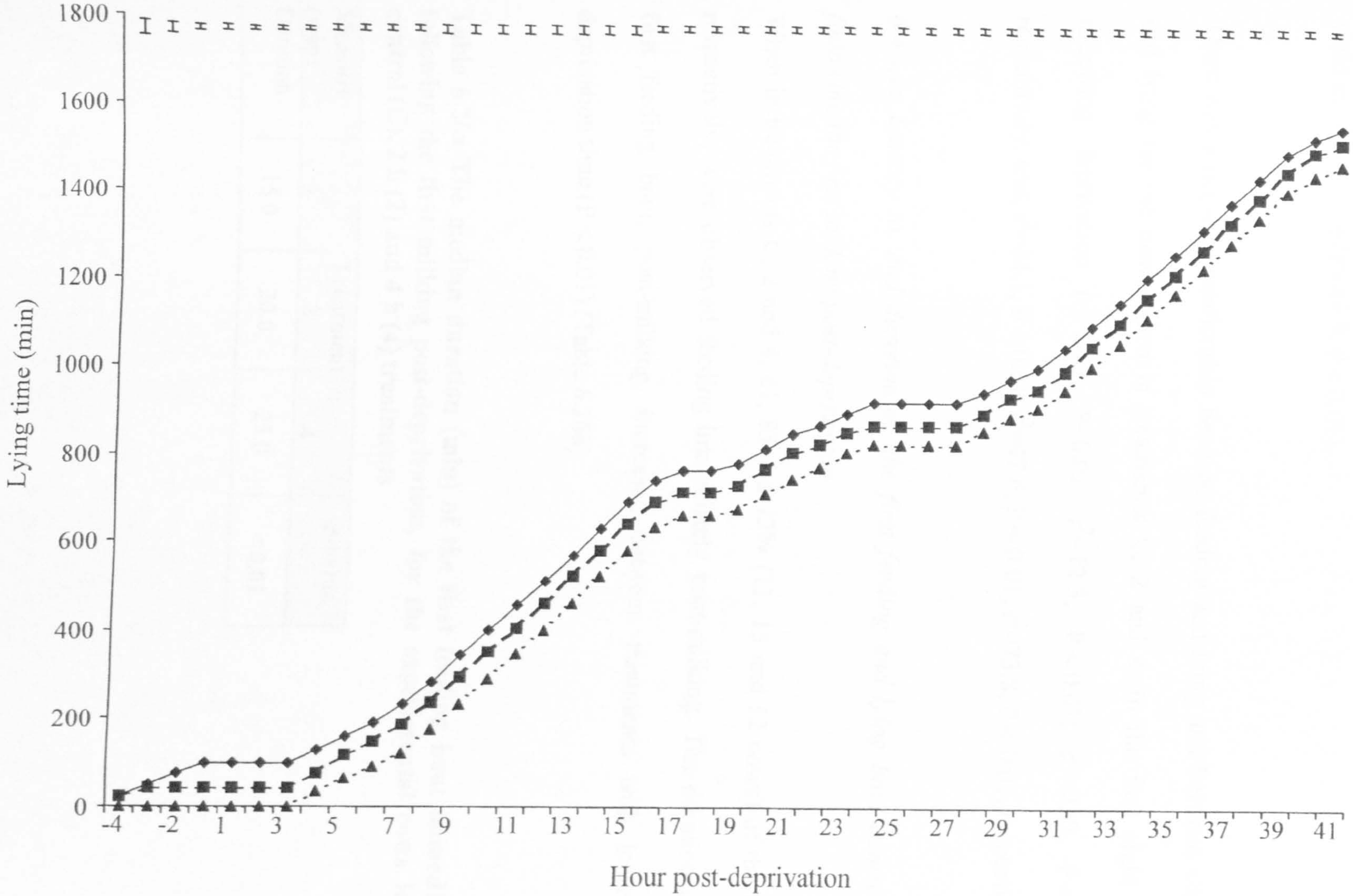


Fig 6.6a The accumulated lying time (min) per hour for all the experimental animals in the control ——◆——, 2 h deprivation ----■---- , and 4 h deprivation ·····▲····· treatments post deprivation (hours 1 to 40), and for the four hour deprivation period (hours –4 to –1) (I = 1 SED)

6.4.5.1a Behavioural and behavioural versus production variable correlations for post-deprivation video observations

When the cows were in treatment 2 there was a positive relationship between milk yield and feeding time (r^2=22.6, P=0.03), and a negative relationship between milk yield and lying time (r^2=48.9, P<0.01).

There was a negative relationship between feeding and lying and between standing and lying for the cows when in treatments C, 2 and 4, in the first eight hours following deprivation (r^2=28.3, P=0.01; r^2=72.3, P<0.01; r^2=40.0, P<0.01, respectively, and, r^2=84.1, P<0.01; r^2=47.6, P<0.01; r^2=73.8, P<0.01, respectively).

6.4.5.2a Latency to, and duration of, the first feeding and lying bout immediately following the first milking post-deprivation

When in treatments C, 2 and 4, 61, 83 and 72% (11, 15 and 12 cows) of the cows, respectively, were observed feeding immediately post-milking. The duration of the first feeding bout, post-milking, increased between treatments with increasing deprivation time (P<0.01) (Table 6.26a).

Table 6.26a The median duration (min) of the first feeding bout immediately following the first milking post-deprivation, for the experimental cows in the control (C), 2 h (2) and 4 h (4) treatments

Measure (min)	Treatment			P-value
	C	2	4	
Duration	15.0	20.0	25.0	<0.01

There was no significant difference between treatments in the latency to the first lying bout post-milking (Table 6.27a). Thus, cows during all treatments took approximately 20 to 30 min to lie down following the first milking, post-deprivation. There was a tendency for the cows when in treatments 2 and 4 to have a longer first lying bout, post-milking, compared to treatment C (P=0.07) (Table 6.27a).

Table 6.27a The median latency (min) to, and the mean duration (min) of the first lying bout immediately following the first milking post-deprivation, for the experimental cows in the control (C), 2 h (2) and 4 h (4) treatments

Measure (min)	Treatment C	2	4	SED ±	P-value
Latency	30.0	23.3	29.0	-	0.41
Duration¶	36.7 (1.565)	64.9 (1.812)	52.1 (1.717)	(0.1026)	0.07

¶ Used back transformed data to calculate values. Transformed values in parenthesis.

Previous treatment had no significant effect on the mean duration of the first lying bout immediately following the first milking, post-deprivation (P>0.10).

6.5a Discussion

6.5.1a Milk yield recordings

Two to four hours of lying and feed deprivation had a negative impact on milk yield. Mean milk yields tended to be lower (c. 0.6 kg cow^{-1}) for the cows when deprived for two to four hours, compared to no deprivation, during the second milking post-deprivation, but not for the subsequent milkings. As milk yield was not affected until the second milking (17 h post-deprivation), it is likely to have been caused by feed deprivation rather than the stress of forced standing. If forced standing was having an effect, it is expected that this would have been observed during the first milking, *i.e.* immediately after deprivation (Chapter 5a). Here, stress hormones may have inhibited the release of oxytocin, which plays an important role in milk let down by constricting the muscles surrounding the alveoli to excrete milk (Phillips, 2001). However, milk yield appeared to be affected by a slower acting, longer-term response. It is proposed that milk yield was affected by alterations in milk synthesis rather than milk let down, due to feed restriction. A reduction in feed intake would have resulted in fewer circulating nutrients within the blood being available for milk production. Fibre and starch, the breakdown of which produce the volatile fatty acids propionate, acetate and butyrate are transported via the blood stream to the mammary gland, and utilised by the mammary synthetic cells for milk synthesis (Phillips, 2001). A reduction in these volatile fatty acids would slow the process of milk synthesis. A reduction in milk yield has also been observed in other studies whereby dairy cows have been restricted from feeding (Verkerk *et al.*, 1999; Auldist *et al.*, 2000). Verkerk *et al.* (1999) reduced feed intake to 75% of the recommended level for milk production for seven days and recorded a similar reduction in milk yield to that of this study, although this difference was not significant (15.6 versus 15.1 kg milk, sem 0.3). This

non-significant result may have been due to the cows having access to a paddock for three hours per day, thus allowing them to compensate for some of the feed restriction by grazing. In addition, Verkerk *et al.* (1999) observed that the cows fed to the recommended level for milk production consistently left some of their ration, which may suggest that the restriction calculation of 75% was over-estimated, *i.e.* in real terms, the deprived cows were being restricted by less than this calculated amount.

The experimental design may have been responsible for the absence of an immediate reduction in milk yield, which was observed in a previous lying deprivation study (Chapter 5a). In the current study, each cow underwent the experiment three times. On the second and third time through the test, the cows may have known something about the deprivation procedure that was to be imposed, as they would have either experienced or observed it before. A knowledge of what was going to happen, *i.e.* expectation, may have enabled the cows to psychologically cope better with the situation and thus be less stressed (Van der Harst, Kapteijn & Spruijt, 2002; Veissier, Boissy & Desire, 2002). Such psychological adaptation, possibly along with some physical adaptation, may have also been responsible for milk yields not to be significantly affected by repeated lying deprivation in other studies (Munksgaard & Lovendahl, 1993; Verkerk *et al.*, 1999).

An unexpected finding was that the mean morning milk yields for the cows when in treatment 2 were non-significantly lower than those recorded when in treatment 4. During the first few hours post-deprivation the cows in treatment 2 initially spent less time feeding, but more time lying, compared to when in treatment 4 (see sections 6.5.7a & 6.5.8a). This increased time spent feeding when in treatment 4 may have

provided an increased supply of nutrients, which was enough to slightly increase milk yields above those of treatment 2. The difference in time spent feeding between the deprivation treatments during this period may have been small and not statistically significant, but feeding time does not necessarily reflect level of intake. Cows can also employ alternative methods to increase feed intake as well as extending their grazing time. For example, studies that have manipulated the intake levels of dairy cows or subjected them to a period of fasting have observed an increase in biting rate whilst feeding (Manning, 1972; Greenwood & Demment, 1988; Dogherty *et al.*, 1987 & 1989; Patterson *et al.*, 1998). Similarly, pigs fed a restricted ration were observed chewing food at a greater rate than non-restricted pigs (Day *et al.*, 1995). The decrease in milk yield with time was expected, as after an initial peak in early lactation milk yield declines as lactation progresses (English *et al.*, 1995).

6.5.2a Continuous recordings

Leg raising, repositioning and weight shifting behaviours all increased in frequency with time spent in forced standing and feed deprivation. In addition, butting was primarily observed in those cows being deprived of lying and feeding. Weight shifting in response to lying deprivation in dairy cows has also been observed in other studies (Ruckebusch, 1974; Hopster *et al.*, 2002). The combined behaviours, shifting, restlessness- and stress-related behaviours, showed the same pattern of increase, presumably because they were formed from combinations of the individual behaviours above. This suggests that the cows tended to become more frustrated with increasing deprivation of food and lying. Behaviours such as leg raising, repositioning and weight shifting may enable the cow to cope with forced standing by alleviating strain on the legs and hoofs. Regular movement, or shifting, may temporarily prevent

or reduce discomfort. Butting, on the other hand, is more likely to be a behavioural expression of a cow's subjective feelings. This behaviour has previously been shown to be expressed in dairy cows in response to food deprivation (Sandem *et al.*, 2002). Similarly, hens and pigs become more aggressive when they are prevented from feeding (Duncan & Wood-Gush, 1972; Kelley, McGlone & Gaskin, 1980; Webster, 2000). Researchers suggest that aggression, such as butting, can be performed as a result of frustration (Dollard *et al.*, 1939; Miller *et al.*, 1941; Scott, 1948) and is an animal's attempt to cope with a negative situation (Sandem *et al.*, 2002). Frustration, in this case, could have arisen as a result of not being able to lie down, only being able to lie on a relatively uncomfortable surface, due to the negative impact of forced standing and/or being deprived of food. As the deprived cows could see the other cows eating this may have also caused them to become more frustrated. In addition, the increased butting activity may have partly been due to being confined during deprivation, as animals have been shown to be more aggressive when space allowance is reduced (Grigor *et al.*, 1997; Grasso *et al.*, 1999). As the frequency of these behaviours increased with deprivation time this may suggest that the negative physical and psychological effects of lying and feeding prevention, and the motivation to lie and feed, are cumulative. This would imply that the longer the deprivation period, the more severely an animal's welfare is compromised. It is also possible that the increased frequency of the behaviours, to some extent, could be attributed to boredom, as animals housed in barren environments have been shown to be more active (Stimulus Response, 1995).

When in treatment 2 the cows increased their frequency of leg raising, weight shifting, shifting, restlessness- and stress-related behaviours during actual deprivation,

which further confirms that such behaviours are increased as a result of being prevented from lying and feeding. As there was no significant difference between hours in the performance of these behaviours for the cows when in treatment 4, this could suggest that the negative effects of lying and feeding deprivation were immediate and that the cows were unable to adapt to the situation within the four hour period.

In treatment 4 the increase in frustration-related behaviours in the second and proceeding hours, compared to the first, indicates an initial increase in this behaviour with time, which was largely due to head swinging behaviour. Head swinging has been shown to be performed by dairy cows, horses and chickens experiencing frustrating and stressful situations (Sandem *et al.*, 2002; Cook, 1992; Duncan & Wood-Gush, 1972, respectively). Therefore, the increase in head swinging activity in the second hour by the treatment 4 cows suggests that they may have been more frustrated during this time. It would appear that the second hour of deprivation may have been more frustrating due to a time of day effect, rather than a cumulative one, as this increase was not observed in the second hour of deprivation in treatment 2. During the second hour of the deprivation period the cows may have been more motivated to lie down, which, because being prevented from doing so, made them more frustrated and thus they increased head swinging activity. This may be supported by the observation that when in treatment C the cows spent more time lying, but not feeding, in the second and third hour of the deprivation period compared to any other hour. Head swinging in cows in treatment 4 decreased in the third and fourth hour, to that observed in the first hour, possibly because the cows

adapted to this stress, rather than expressing it through alternative behaviours, as no other frustration-related behaviour was observed to increase during this time.

The observer noted that the deprived cows would nose the ground where feed was usually presented. In doing this, it appeared that the cows were attempting to obtain food in some way, possibly by searching for small patches of food where it had been scraped away. As this behaviour was only observed by the cows undergoing deprivation, and was not observed during a previous study whereby cows were only deprived of lying, it is reasonable to suggest that this activity appeared to reflect feeding motivation. Nosing the ground may be equivalent to beak-wiping that has been observed in chickens when being prevented from feeding (Duncan & Wood-Gush, 1972; Preston, 1987). In an experiment by Preston (1987), chickens were denied access to food by placing covers over the feed troughs. During periods of restricted access the chickens were observed "rubbing or nuzzling" their beaks on the cover. The motivation to feed may have remained constant for the duration of the deprivation period, as the frequency of this behaviour did not significantly differ between hours during this time.

The increase in leg raising, repositioning, weight shifting, butting and nosing the ground indicate that the cows became more active with deprivation. Increased activity in response to food deprivation has also been observed in chickens (Duncan & Wood-Gush, 1972; Preston, 1987; Savory & Maros, 1993; Webster, 2000), turkeys (Hocking, Maxwell & Mitchell, 1999) and pigs (Kelley, McGlone & Gaskin, 1980; Day *et al.*, 1995). Typically, the responses observed were increased pacing and non-nutritive pecking in fowl, and increased aggression and rooting behaviour in pigs. It is

expected that the increased activity is more related to the frustration being experienced, rather than food seeking behaviour, since such behaviours have been observed when animals have been presented with frustrating situations. For example, the behavioural responses observed in this experiment were the same as those when the cows were deprived of lying only (Chapter 5a). In addition, food restriction alone has not been observed to affect locomotion in pigs (Day *et al.*, 1995).

The increase in ground sniffing behaviour, between treatments, with increasing deprivation time, is likely to be related to the requirement to lie down. The experimenter observed this behaviour taking place immediately prior to lying when the cows were in treatment C. It appeared that this activity was performed to select or assess a suitable lying area. It is likely that the deprived cows executed this appetitive behaviour more frequently, as they wanted to, or considered, lying but were prevented from doing so. This may be supported as in treatment 4 the cows ground sniffed more frequently during the second hour of deprivation, which coincides with the most time spent lying in treatment C. This behaviour was also observed in cows undergoing a lying deprivation experiment (Chapter 5a). Sniffing behaviours are a combination of nosing the ground and sniffing the ground and thus have been considered separately above.

Over the entire deprivation period, the cows rubbed their heads against the housing more frequently and for longer, and interacted with the housing more frequently, the longer they were deprived of lying and feeding. This finding is in agreement with other studies whereby cattle deprived of lying also interacted with their surroundings more frequently, *i.e.* licking or chewing stall fixtures (Munksgaard & Simonsen,

1996; Munksgaard *et al.,* 1999). It is plausible that these behaviours were performed to compensate for a lack of stimulation within the deprivation environment (Kerr & Wood-Gush, 1987; Stimulus Response, 1995; Munksgaard & Simonsen, 1996; Hansen & Berthelsen, 2000), rather than as a direct result of the stress of lying prevention. This may be supported, as these behaviours were not significantly different between deprived and non-deprived cows in an earlier lying deprivation experiment (Chapter 5a). Although the cows in this previous experiment were under similar conditions, they had the opportunity to feed, which provided some additional stimulation from feeding itself and from those activities that occur at the feed barrier. Head rubbing and housing interaction may be less likely to be observed if cows were not confined, or were deprived of lying whilst at pasture.

In treatment 4 the cows drank for significantly less time per bout compared to when in treatments C and 2. This is in line with other studies that have also recorded animals spending less time drinking when deprived of food [Preston, 1987 (chickens); Day *et al.*, 1995 (pigs); Cockram *et al.*, 1999 (sheep)]. In addition, Savory (1978) observed a positive relationship between food and water intake in hens, with feed intake declining when daily water supply was restricted. It may be that additional water is required whilst eating, possibly to clear the mouth of debris after feeding. This could be supported by the observation that when in treatment C the cows spent most time drinking in the first hour, which is also when they spent most time feeding. Contrary to this finding, Savory and Maros (1993) observed that feed restricted chickens spent more time drinking in comparison to those fed *ad libitum*. However, this increased drinking time mainly took place after the chickens were fed, which would therefore support the current finding. The cows may have maintained the same frequency of

drinking per hour of the deprivation period when in treatment 4, compared to treatment C, due to the possible stress experienced from forced standing. Polydipsia has been shown to occur during periods of stress in other animals (Tarjan & Denton, 1991; Rodriguez de Turco *et al.*, 1993; Howell *et al.*, 1998).

There was no significant difference between treatments in the frequency and time spent self grooming and the frequency of body care and licking behaviours. This is contrary to the findings of other studies whereby cattle deprived of lying groomed themselves more often and increased the frequency of licking behaviours (Munksgaard & Simonsen, 1996; Munksgaard *et al.*, 1999). This discrepancy is most likely due to differences in experimental design, since Munksgaard and Simonsen (1996) and Munksgaard *et al.* (1999) both deprived cows of lying for 14 hours per day for several weeks, whilst the present experiment was considerably shorter. In addition, the orally-centred behaviours observed by Munksgaard *et al.* (1999) were not observed to increase until the third week of deprivation.

During the deprivation period cows in treatment 4 decreased the frequency and duration of self grooming and the frequency of body care and licking behaviours. Orally-centred body care activities such as these have been shown to be induced by stress in animals (Van Erp *et al.*, 1994; Hansen & Berthelsen, 2000; D'Aquila *et al.*, 2000). This suggests that the cows may have adapted to the stress slightly during the deprivation period. This may be supported as the display of frustration-related behaviours peaked in the second hour and then declined slightly in hours three and four. In addition, Munksgaard and Simonsen (1996), when depriving cows of lying recorded an increased concentration of ACTH in the first hour of the deprivation

period. This increased stress response may have been responsible for the higher frequency of self grooming, body care and licking behaviours in the first hour of deprivation for the cows when in treatment 4.

6.5.3a Behavioural versus production variable correlations for continuous recordings

As the cows came closer to parturition they leg raised and shifted more frequently and increased the performance of restless- and stress-related behaviours, in treatments C and 2, and weight shifted and tended to perform frustration type behaviours more frequently, when in treatment C. This is expected to be due to the pressure of the foetus causing some discomfort whilst standing, and possibly also putting extra strain on the limbs, which may have been eased by regularly shifting body weight and moving the legs. A similar reasoning may also be used to explain why in treatment C the cows increased all the above behaviours, except frustration, in response to increasing milk yield. These behaviours may have been employed to reduce possible strain on the legs caused by an additional milk load, possibly whilst the cows were feeding. The high yielding cows may have also walked less frequently for the same reason, as an increased milk load or udder size may have made this activity uncomfortable or awkward. The tendency for the cows, in any of the three treatments, to increase head swinging with milk yield may demonstrate the frustration experienced by this increased strain on the limbs. Significant correlations between some of these behaviours and these two production variables were not observed in treatments 2 or 4, possibly because they were performing these behaviours anyway due to the effect of lying deprivation, and therefore regardless of milk yield and stage of pregnancy. Similarly, this may also be the reason for weight shifting not to be significantly correlated with body condition score for treatments 2 or 4.

When in treatment C the thinner cows weight shifted more often than the fatter cows. Wildman *et al.* (1982) reported an inverse relationship between milk yield and body condition score in dairy cows, and Veerkamp *et al.* (1994) observed that higher yielders have a significantly lower mean body condition score in comparison to lower yielding cows. This relationship was tested and there was a negative correlation between these variables for the cows (r^2=38.3, P=0.01). Thus the thinner cows were higher yielding, and leg raising behaviour may have been performed to reduce discomfort caused by the extra stress on the legs due to an increased volume of milk within the udder. Furthermore, this may also explain why the thinner cows, when in treatment 4, head swung more frequently, as head swinging increased with milk yield.

6.5.4a Behavioural correlations for continuous recordings

It is assumed that the cows increased leg raising with weight shifting when in treatment C as the function of their performance was similar, *i.e.* they both potentially relieve strain on the limbs.

Head swinging may be expected to increase with leg raising and shifting for the cows when in treatments C and 2 because they may both be behaviours related to discomfort and frustration. This may be further supported as the incidence of leg raising increased with the performance of frustration-related behaviours.

Ground sniffing was positively correlated with frustration-type behaviours for the cows when in treatment 2, and stress-related behaviours in treatment 4. Sniffing behaviours were positively correlated with frustration- and stress-related behaviours for the cows when in treatment 2, and stress-related behaviours only in treatment 4.

This may be because the cows may have wanted to lie down and so sniffed the ground, but being deprived of doing so may have caused them to become frustrated and stressed. Ground sniffing was positively correlated with leg raising and restlessness type behaviours in treatment 4, and sniffing behaviours were positively correlated with leg raising, head swinging (treatment 2 only), repositioning (treatment 2 only), shifting and restlessness type behaviours for the cows when in both deprivation treatments. As ground sniffing and sniffing behaviours were correlated with behaviours that can be regarded as possible indicators of frustration and stress, this may further support the proposed relationship. Frustration- (treatments 2 & 4), restlessness- and stress- (both treatment 2) related behaviours all increased the more the cows interacted with the housing, which is probably because this behaviour is a function of sniffing the housing. It is thus assumed that housing interaction increased with leg raising, head swinging (both treatment 2) and ground sniffing (treatments 2 & 4) as the origin of their performance is similar, *i.e.* they are all potential indicators of frustration.

Self grooming, body care and licking behaviours were all positively correlated with frustration-related behaviours for the cows when in treatment 2. Munksgaard and Simonsen (1996) also demonstrated that cows increased grooming activity when deprived of lying, indicating frustration. Other studies have also supported a relationship between increased grooming and stress in animals (Van Erp *et al.*, 1994; Hansen & Berthelsen, 2000; D'Aquila *et al.*, 2000). In addition, rats have been shown to excessively groom in response to the stress hormone adrenocorticotrophin (Van Erp, Kruk & De Kloet, 1993).

Body care and licking behaviours were observed to increase with increasing head swinging for the cows when in treatment 2, which would be expected if these behaviours formed a set of similar actions in response to a frustrating situation. Similarly, this may be why body care and sniffing behaviours increased together in treatment 2. However, these behaviours were also positively correlated whilst the cows were in treatment C. This may be explained as the frustration experienced by the cows whilst being deprived may be different, and have arisen from a different source, to that experienced during control conditions. In treatment C, it may have been a lack of stimulation within the environment that caused the cows to become frustrated (Stimulus Response, 1995). During deprivation, the cows' frustration may have arisen primarily from being forced to stand and/or the thwarting of feeding behaviour. Different experiences, or sources, of frustration may give rise to similar behavioural displays.

It is possible that when in treatment 2 the cows spent longer rubbing their heads against the housing the more they sniffed the ground and performed sniffing behaviours, as this behaviour may be frustration driven. During deprivation, the cows may have sniffed the ground because they wanted to lie down or possibly feed, but the prevention of these behaviours induced frustration, which manifested itself as head rubbing behaviour. This may be further supported as head rubbing could be categorised as a body care activity (Phillips, 1993), which was observed to increase the more the cows performed frustration-related behaviours when in treatment 2.

Nosing the ground increased with sniffing behaviours in treatment 4. It seems that this predominantly took place whilst the cows were sniffing the ground, rather than the housing, as there was only a positive correlation between this behaviour and nosing

the ground. It is likely that these behaviours took place during the same time: If the cows were nosing the ground in an attempt to assess an area for food, sniffing, also, would aid this appraisal.

The tendency for the cows, when in treatment 2, to reposition themselves more frequently the more they nosed the ground may have been so that they could gain a better position to perform this behaviour.

As grooming, head rubbing, and housing interaction have all been shown to be possibly induced by frustration, then it is reasonable to suggest that nosing the ground may have been causing some frustration as these behaviours increased the more the cows performed this behaviour in treatment 4. The deprived cows were likely to have sniffed the ground in an attempt to feed, but as this behaviour was not fulfilled the cows became frustrated and thus performed these body care type behaviours.

Repositioning increased the more frequently the cows were observed walking, when in treatment 2. This is because repositioning substituted for walking during the deprivation period. Maybe those cows observed walking more frequently were more affected by the limited space allowance and thus more readily redirected this behaviour to repositioning. It is expected that some physical discomfort came from the limited area in which the cows could move, as opposed to just being deprived of lying.

6.5.5a Instantaneous recordings

When in treatment 4 the cows primarily replaced lying and feeding with standing (not ruminating) in all hours except the third, where they spent more time standing ruminating. The increased time spent standing ruminating in this hour may be due to a time of day effect, as the cows spent more time in this activity when in treatment 2, and most time lying ruminating in treatment C, also at this time. Between c. 0900 and 1300 h, ruminating activity has been shown to be at its highest at about mid-day in grazing dairy cows (Phillips, 1993), which may support this suggestion. In contrast, in treatment 2 the cows spent more time standing ruminating than just standing in both deprivation hours. This may be because the opportunity to ruminate was greater due to the additional time spent feeding in the first two hours of the deprivation period. Munksgaard and Simonsen (1996) also noted that cows spent the majority of their time standing ruminating when deprived of lying.

It appears that cows prefer to ruminate whilst lying, as standing ruminating was rarely observed over the entire deprivation period for the cows when in treatment C, and in the first two hours in treatment 2. This is supported by Phillips (1993), who states that rumination often takes place during periods when cows are less alert, thus is likely to be associated with periods of rest. Additionally, in lactating dairy cows, standing ruminating only typically takes place for approximately 0.04% of the day in comparison to 20 - 25% for lying ruminating (Phillips, 1993). The increase in time spent standing ruminating, between treatments, with increasing deprivation time would therefore be expected to be related to the deprivation of lying, rather than feeding. This may be supported by the observation that this trend was observed in the previous lying deprivation study (Chapter 5a). These results are also in agreement

with those of Munksgaard and Simonsen (1996) and Munksgaard *et al.* (1999), who noted that lying deprived cows spent a greater proportion of their time ruminating standing in comparison to control cows.

The proportion of time spent ruminating, both lying and standing, was approximately the same for the cows when in treatments C, 2 and 4 (41, 45 and 42 %, respectively). However, in the previous lying deprivation experiment, the time spent ruminating decreased, between treatments, with increasing deprivation time. It is expected that this was due to the cows having the opportunity to feed in the latter experiment. In the present study, the deprived cows could only stand or stand and ruminate. Ruminating whilst standing may be preferable to standing only, as this provides the cows with an activity to perform and also some stimulus.

In treatment C the cows were observed lying and feeding for approximately equal amounts of time in each hour of the deprivation period for a total of 26 and 59 min, respectively. In treatment 2 the cows were also observed lying and feeding for approximately equal amounts of time in the first two hours for a total of 14 and 33 min, respectively. This indicates that the cows were motivated to both lie and feed throughout the entire deprivation period. Although the cows were deprived of lying for four hours in treatment 4, the cows only spent a mean time of 100 min in lying behaviours (lying & lying ruminating), when in treatment C. Thus, in treatment 4 the cows were, in real terms, only deprived of lying for this amount of time. Likewise, when in treatment 2 the cows were only deprived lying for 45 min. Similarly, when in treatments 2 and 4 the cows were only actually deprived of feeding for 26 and 59 min, respectively.

6.5.6a Behavioural versus production variable and behavioural correlations for instantaneous recordings

When in treatment C, it is possible that the high yielding cows spent longer standing ruminating and less time lying ruminating, as the former behaviour could be more easily interspersed with periods of feeding. Cows typically ruminate for approximately 45 min per bout, but high yielding cows will reduce this activity in order to perform other behaviours, such as feeding (Phillips, 1993). When in treatments C and 2, the cows spent more time standing and less time lying as milk yield increased. Chaplin and Munksgaard (2001) and Fregonesi and Leaver (2001) also recorded a negative relationship between milk yield and lying. Similarly, other researchers have noted that the time spent lying increased with the progress of lactation (Veris *et al.,* 1980; Phillips & Leaver, 1985b). This may support the negative relationship between lying and milk yield, as, after an initial peak in early lactation, milk yield declines with the progress of lactation (English *et al.*, 1995). The increased standing time may have been spent at the feeding barrier. This may be supported, as all standing and feeding behaviours increased with milk yield. There was also a positive relationship between feeding and standing ruminating for the cows when in treatment 2. Other studies have also revealed a positive relationship between feeding time and milk yield (Lathrop *et al.,* 1988; Phillips & Denne, 1988; Bao *et al.,* 1992; Gibb *et al.,* 1999). All of the behaviours correlated with milk yield above took the opposite trend when correlated with body condition score, when the cows were in treatment C. It is likely that those factors responsible for the correlation between a behaviour and milk yield are also responsible for those between the behaviour and

body condition score. This is because there was a strong negative relationship between body condition score and milk yield (r^2=38.3, P=0.01).

Feed intake declines as pregnancy advances, and rumen capacity declines as a result of the presence of the foetus (Phillips, 1993). Thus, cows further into pregnancy may ruminate more frequently as gut fill is achieved more quickly, which may account for the positive relationship between the frequency of ruminating while standing and the number of days to parturition in treatment C. This additional time spent standing ruminating may be responsible for the tendency for lying ruminating to decrease as the number of days to parturition advanced. In addition, those factors responsible for the positive correlation between standing ruminating and milk yield may also be responsible for the correlation between this behaviour and the number of days to parturition, as there was a strong positive relationship between these two production variables (r^2=54.9, P=0.00).

As milk yield increases from lactation one to eight (Phillips, 2000b), it may be that higher yielding cows have a greater feed intake and thus spend longer periods ruminating, which would account for the positive relationship between lactation number and lying ruminating for the cows when in treatments C and 2. The tendency for the cows when in treatment 2 to decrease standing time with lactation number could be due to this behaviour being replaced by lying ruminating.

The time spent feeding and standing were negatively associated for the cows when in treatment 2, indicating a trade-off between these activities, presumably because these cows were not prepared to reduce lying time to increase their time spent feeding.

6.5.7a Lying positions and changes in posture post-deprivation

It is possible that cows determine a probable comfortable lying position prior to lying, as changes in posture were not observed in any of the treatments once the cows had lain down. Alternatively, changing posture may be difficult to perform once lying and, unless particularly uncomfortable, the cow may settle in the adopted position. It is assumed that the lateral lying posture was preferred over the sternal lying posture, as only one cow was observed in the latter.

Although it was not possible to test statistically, there seemed to be a switch to left side laterality in the deprived cows when lying. This may have been a more comfortable side to lie due to the presence of the foetus being on the right-hand side of the body (Phillips, 1993). The cows may have become more aware of its presence due to the additional time spent standing causing some discomfort.

When in treatments 2 and 4 there was a tendency for fewer of the cows to lie during the first 45 min immediately following deprivation, compared to treatment C. During this period the deprived cows that were not lying were observed predominantly feeding, and the time spent feeding tended to increase with deprivation time. It is therefore reasonable to suggest that the motivation to feed exceeded that to lie in these cows immediately after being deprived of both, and this was greater in those cows deprived for four hours.

6.5.8a Post-deprivation video observations

During the first eight hours post-deprivation, compared to treatment C, in treatments 2 and 4 the cows increased their feeding time and not the time spent standing or lying,

indicating that the motivation to feed was greater than that to lie after being deprived of both. This is in agreement with Ruckebusch (1974) who also found that cattle chose to feed immediately after being deprived of lying and feeding for 14 and 22 h day^{-1}. The prevention of feeding when in treatments 2 and 4 resulted in the greatest increase in feeding time, compared to treatment C, during the first eight hour period, in comparison to any other eight hour period, post-deprivation. During this time the cows when in treatments 2 and 4 compensated for about 94% (31 min) and 47% (28 min), respectively, of their actual deprived feeding times. This rebound indicates that the need to feed builds up significantly after only a few hours of deprivation. This is demonstrated further as the motivation to feed in the first feeding bout post-milking also increased with increasing deprivation time. In addition, although the latency to the first lying bout post-milking was approximately the same for the cows during all treatments, the proportion of this time spent feeding was greater for the cows when in treatments 2 and 4, compared to treatment C (86, 86 & 50%, respectively).

The initial rebound in feeding time primarily took place during the first few hours post-milking. This time period may be set by the feeling of satiety, which may be related to the physical capacity of the stomach, as cows and pigs deprived of feeding for considerably longer periods (14 & 20 h, respectively) also only spent two to three hours in increased feeding activity immediately post-deprivation (Ruckebusch, 1974; Beattie *et al.*, 2002). Interestingly, when in treatments 2 and 4 the cows fed for approximately the same amount of time (c. 30 min) during the first eight hour period. It was expected that the cows would have fed for longer when in treatment 4. There are two possible reasons for why they did not: First, in treatment 4 the cows may have increased their rate of feed intake by eating faster and/or increasing their bite size, as

has been observed in dairy cows (Manning, 1972; Greenwood & Demment, 1988; Dogherty *et al.*, 1987 & 1989; Patterson *et al.*, 1998), sheep (Iason *et al.*, 1999) and pigs (Day *et al.*, 1995) after a period of fasting; and second, the motivation to lie may have exceeded that to feed after a short period of feeding.

In treatment 2, the cows fed for c. 16 min more than when in treatment C, between hours 25 and 32 after deprivation. This increased feeding time primarily took place during the same times as those immediately post-deprivation, *i.e.* 17.20, 19.20 and 20.20 h. It would seem, then, that the cows increased their feed intake because they had a long feeding bout at these times the day before. However, when in treatment 4 the cows did not show this response, and this was not due to them spending more time lying. This result may be a spurious finding, or may relate to an increased feeding rate in these cows.

Over the 41 h period, when the deprivation period was taken into account, the cows spent longer feeding when in treatment 2 than 4. This may reflect the difference in the coping strategies employed by the cows when in the two treatments. The previous lying deprivation experiment revealed that two hours of lying deprivation seemed to have little effect on the motivation to lie, post-deprivation (Chapter 5a). This may mean that the cows would not feel they had to increase their rate of feed intake to make time to lie down. Therefore, to compensate for the deprivation period, they increased their feeding time instead and spent c. 61 min more time feeding over the 41 h period, compared to when in treatment C. In contrast, the motivation to lie after four hours of deprivation was shown to be greater, but as the cows in this study were more highly motivated to feed post-deprivation they reduced the amount of time spent in

this activity and may have compensated by increasing their rate of intake. Thus, over the 41 h, they may have achieved a greater level of intake compared to when in treatment C, but within a similar amount of time.

Over the entire 41 h period, including the deprivation period, the lying times for the cows in all three treatments were similar. Correspondingly, Hopster *et al.* (2002) observed that three hours of lying deprivation had no effect on the lying times of dairy cows over a 24 h period, but cows deprived of six hours lost 106 min of lying time, compared to the control cows. In contrast, when Metz and Wierenga (1984) deprived cows of lying and feeding for three hours, the cows compensated for 53% of their reduced lying time within seven hours, demonstrating some rebound behaviour. A possible explanation for their result is that the cows may not have been motivated to feed during the deprivation period. This was difficult to determine since they did not report how much feeding time was lost during deprivation.

Given that there was no significant difference between the lying times for the cows when in the deprivation treatments, compared to treatment C, for any of the eight hour periods, this suggests that the deprived cows gradually increased their lying time over the 41 h period, by small increments, rather than as a rebound. This was also observed by Metz and Wierenga (1984) who found that cows deprived of lying for six hours per day compensated for this by increasing their lying time over the remaining parts of the day. The lying times reported here were similar between treatments for each eight hour period, except the third, indicating that this behaviour was not compromised to increase feeding time. Whereas, although not significantly different, the time spent standing was generally lower for the cows when deprived compared to

when in treatment C in each period. In addition, as there was no significant difference in standing time between treatments over the 41 h period when the deprivation period was included, then the deprived cows must have reduced this behaviour over the 41 h. The reduction in lying time between hours 17 and 24, post-deprivation, by the cows when in treatment 2, was mainly due to spending more time feeding, possibly as a continuation of their strategy to increase feed intake.

In treatment 2, it took 24 h for the cows to obtain approximately the same feeding time as when in treatment C, thus achieving 100% feeding time compensation. In treatment 4, the cows were unable to match the feeding time of that when in treatment C or 2 within the 41 h period. This is probably due to the cows being unwilling to compromise any more of their lying time so as to increase feeding time. This may indicate a trade-off between these behaviours (see below). This may also be supported by Jensen *et al.* (2002) who recorded that cows would work in an operant conditioning test to lie for approximately 13 h d^{-1}, and Wierenga and Hopster (1990) observed cows lying for the same amount of time in a cubicle system. Also, in the previous lying deprivation study (Chapter 5a) the control cows were observed lying for 14.8 h d^{-1}. These values are close to the lying time of 13.8 h d^{-1}, which was observed for the cows in this study when in treatment 4. Alternatively, they may have been able to increase their feed intake rate, rendering an increased feeding time unnecessary.

In treatment 2 the cows did not increase their lying time to match that when in treatment C, and when in treatment 4 the cows did not increase their lying time to match that when in treatment 2. However, in treatments 2 and 4 the cows increased

their lying time at the same rate as when in treatment C, over the 41 h period, suggesting that they value this activity and were not prepared to compromise it further by increasing feeding time. When in treatment 4, the cows may have been unable to achieve complete lying compensation as there was little else they could give up to achieve this. This is likely, as in the previous lying deprivation study (Chapter 5a), cows deprived of four hours lying achieved approximately the same lying time as those deprived of two in 29 h, but could only do this by reducing their feeding time.

In treatment 2, it appears that the cows increased their feeding time at the expense of fully recovering their lost lying time. Two hours of lying deprivation may not be severe enough to motivate the cows to decrease their lying deficit (Chapter 5a). Other studies concerned with the deprivation of lying in dairy cows have also observed incomplete compensation (Munksgaard *et al.*, 1999; Munksgaard & Simonsen, 1996; Metz, 1985). Metz (1985) suggested that a 100% recovery rate is unlikely to be found as cows deprived of sleeping never make a complete recovery. It is also possible that full compensation may only be achieved over a longer period. Dairy cows deprived of lying for six hours per day for one week took three days to completely compensate for their daily deprivation (Hopster *et al.*, 2002).

The positive correlation between milk yield and feeding time and the negative correlation between milk yield and lying time for the cows when in treatment 2 were also observed during the deprivation period (see section 6.5.6a for an explanation of these relationships). The behavioural correlations demonstrate that the increased feeding time was achieved by decreasing the amount of time spent lying, and standing time was reduced to increase lying time. This advocates the relationship between

feeding and lying, as observed in previous experiments (Chapter 3b & 5a). Several other studies have also shown that animals will trade their lying time to feed when feeding has been restricted (Veris *et al.*, 1980; Muller-Scharze *et al.*, 1982; Dunbar & Dunbar, 1988; Day *et al.*, 1995; Cockram *et al.*, 1999; Fuerst-Waltl *et al.*, 1999; Fregonesi & Leaver, 2001). For example, Metz (1985) deprived cows of feeding for three hours and they compensated for this by reducing their lying time to feed, especially during the first hour post-deprivation. However, in a following experiment, when they deprived the cows of lying and feeding, the cows did not reduce their lying time to feed, which was also observed in this experiment. By not reducing their lying time the cows were in fact demonstrating the importance of this behaviour. If cows did not value this activity highly, then it would be expected that they would have reduced it to increase their feeding time, based on the significance of the trade-off between these behaviours. The cows' need to lie in this study was clear, as the duration of the first lying bout post-milking tended to be longer for the cows when deprived, compared to when in treatment C. This is in line with the results of Hopster *et al.* (2002), who observed longer lying bouts in dairy cows deprived of lying for six but not three hours.

In conclusion, prevention of lying, even for two hours, poses a risk to cow comfort. Behavioural observations during deprivation demonstrated behavioural signs of fatigue, frustration and stress. The negative physical and psychological effects of deprivation were cumulative, indicating a persistently increasing impact on welfare with time. Both two and four hours of lying and feed deprivation resulted in an immediate feeding rebound post-deprivation, demonstrating that feeding can be regarded as an important and highly motivated activity. Although no such rebound

was observed for lying, this behaviour was not reduced to extend feeding time, as observed when animals have been deprived of feeding only and as would be expected considering the negative relationship between these behaviours. This suggests that lying is also an important and highly motivated behaviour, which could cause suffering if prevented. In addition, whether the cows increased their rate of feed intake and/or reduced their feeding time to maintain a fixed lying time, both strategies highlight the importance of lying after only four hours of deprivation. Two to four hours of lying and feed deprivation also tended to reduced milk yields by approximately 0.6 kg cow^{-1}, further illustrating the importance of these activities. The findings of this study would suggest that preventing cows of lying or feeding, even for a short period, should be avoided.

The priority to feed, rather than lie, post-deprivation, may be related to the timing of the deprivation period. The first eight hours included a post-milking period, when a cow's motivation to feed is usually greater than that to lie (Phillips & Leaver, 1986). Cows returning from milking can postpone lying for c. 1 h in order to feed (Miller & Wood-Gush, 1991; Tyler *et al.*, 1997 & 1998; Overton *et al.*, 2002). If, for example, the end of the deprivation period coincided with a period when the cows were more highly motivated to rest – possibly early morning (Overton *et al.*, 2002) – and did not include a post-milking period, this increased feeding time may not have been so pronounced. It may also be expected that cows would feed after a period of deprivation, since hunger may induce an unparalleled motivational strength to perform this activity, which over-rides other body maintenance type behaviours, except possibly that of thirst (Webster, 1995). Although the motivation to feed can be considered as a useful yardstick, Webster (1995) considers hunger to be the "most

basic, primitive and unremitting of all motivational forces," and, for this reason, it may be too great for this purpose as it is unable to differentiate between lesser motivations (Dawkins, 1983b).

When in treatment 2, the lying time reduction mimicked that experienced by the grazing high yielding dairy cows in a previous experiment (Chapter 3b)(45 versus 61 min., respectively), adding value to the validity of the transference of these results to such cows. However, there are potential confounding factors, which could make a direct comparison implausible. For example, the cows were deprived of lying in one bout. As lying is a polyphasic activity, it is more likely that high yielding cows have a number of shorter lying periods in comparison to their lower yielding counterparts. The experiment also only examined the effects of lying deprivation for one day. It is likely that grazing high yielding dairy cows are deprived of lying every day, possibly for the duration of their lactation. This may lead to cumulative effects, which could not be observed in this study. A further dissimilarity is that the deprivation of lying took place within a small area, which restricted movement. This would not be a situation experienced by grazing dairy cows. It is probable that some of the behaviours expressed, such as leg raising and weight shifting, were symptomatic of standing for long periods in a confined space, rather than being deprived of lying *per se*. The results of this study, in some instances, may therefore be more relevant to the effects of long periods of standing, for example, whilst awaiting veterinary attention or artificial insemination, when confined to concrete yards during periods of wet weather, or due to over-occupation of cubicle housing, on dairy cattle welfare.

6b. The statistical analysis of paired dairy cows II

6.1a Introduction

See introduction for the statistical analysis of paired dairy cows I, Chapter 5b.

6.2b Materials and Methods

6.2.1b Animals, conditions and experimental conditions

The animals, conditions and experimental conditions were as those in Chapter 6a, section 6.2a. The behaviours used for analysis were collected instantaneously during the deprivation period (see Chapter 6a, section 6.2.6a).

6.3b Statistical analysis

All statistical analyses were performed using the 13th release of the Minitab statistical programme (Minitab, 2000). For all of the behaviours only cows from treatment 2 and/or C were used for analysis. Treatment 4 cows were not used, as by the nature of their treatment they were unable to perform certain behaviours, *i.e.* lying, feeding and ruminating lying.

Two methods of analysis were employed. The first method used the cows when they were undergoing either the control or 2 h of deprivation treatment, in the second or third period only. The latter two periods were selected over the first and second, as this maximised any possible period effect. Cows in periods two or three would have been housed together under the experimental conditions either once or twice before, respectively. Therefore, if allelomimicry existed between cows for the behaviours selected, selection of these two periods was more appropriate. If cows experienced each of the desired treatment conditions, *i.e.* one in the second and the other in the

third period, then the treatment condition they were subjected to in the third period was selected, allowing the cows to have been together for the maximum number of times possible. For example, if a pair underwent the control conditions in the second period and 2 h of deprivation in the third period, the data for analysis used for that pair was when they were undergoing 2 h of deprivation. Due to an uneven number of pairs this resulted in four and five pairs of cows undergoing treatment C and 2, respectively, being selected for analysis. The total time (min) spent in each behaviour during the first two hours of the deprivation period was used for analysis. During these two hours the treatment 2 cows were not being deprived of lying or feeding and were therefore under the same conditions as the treatment C cows. Five mutually exclusive behaviours were used for analysis: Feeding, standing, lying, ruminating whilst lying and ruminating whilst standing. To determine the variation between cows within the same pair, *i.e.* cows that were interacting and able to influence each other's behaviour, each cow within each pair was randomly assigned (by coin tossing) to one of two groups for analysis. Hereafter this will be termed the 'within-pair' analysis. This gave rise to nine cows in each group. To determine the degree of variation between cows not within the same pair, *i.e.* cows that could not interact and were unable to influence each other's behaviour, each pair of cows was randomly assigned (by coin tossing) to one of two groups for analysis. Hereafter this will be termed the 'between-pair' analysis. This gave rise to five and four pairs of cows in group one and two, respectively.

The second method used the cows when they were undergoing the control treatment only, and were thus all under the same conditions. This resulted in all nine pairs of cows being selected for analysis from all three periods: Three from the first period,

four from the second and two from the last. The data collected from all four hours of the deprivation period, *i.e.* the total time (min) spent in each behaviour during this period, could therefore be used for analysis. The same five mutually exclusive behaviours were used as in method one. The designation of individual cows and pairs to groups for within-pair and between-pair analysis, respectively, was carried out in the same way as method one. Designation of cows to groups for within-pair analysis resulted in nine cows per group. Designation of cows to groups for between-pair analysis resulted in five and four pairs in groups one and two, respectively.

The random designation of pairs of cows to groups for both methods was conducted to avoid any period effect between groups. For both methods, where pairs of cows were previously housed together, they spent approximately 48 h together under the same conditions on each occasion. The first method maximised the number of times the pairs of cows had previously been together, whilst the second method allowed for a larger data set to be utilised.

All behaviours, for both groups, for within-pairs and between-pairs, and for each method were normally distributed (as estimated by the Kolmogorov-Smirnov test, $P \geq 0.05$). All the data within each behaviour were homogenous (as defined by the F-Test, $P \geq 0.05$). For the within-pair data, for each method, an ANOVA was undertaken for each behaviour between the two groups to calculate the error of the mean square. Similarly, for each method, the GLM was used for each behaviour to calculate this value for the between-pair data, due to the unequal number of cows per group. The error of the mean square provides the best estimate of the population variance (Dytham, 1999). Using this value the standard error of the difference (SED)

between the two groups was calculated for each behaviour for both within-pairs and between-pairs in each method. The coefficient of variation (CV) for both within-pairs and between-pairs, for each behaviour in each method, was calculated using the SED. For both methods, an ANOVA was carried out to compare the within-pairs and between-pairs coefficient values for each of the behaviours analysed. These values followed a normal distribution (as estimated by the Kolmogorov-Smirnov test, $P \geqslant 0.05$) and the data for comparison were homogenous (as defined by the F-Test, $P \geqslant 0.05$). The Kolmogorov-Smirnov analysis was selected over alternative methods to determine whether the observations followed a normal distribution as it is a comparatively more lenient test (Minitab, 2000).

6.4b Results

Table 6.1b shows the coefficient of variation values calculated for each of the behaviours for within-pairs and between-pairs in both methods.

Table 6.1b Coefficient of variation values (%) for between-pairs (BP) and within-pairs (WP) for each behaviour in each method

	Method 1		Method 2	
	Coefficient of variation (%)		Coefficient of variation (%)	
Behaviour	BP	WP	BP	WP
Feeding	12.3	12.1	10.2	11.1
Standing	32.4	29.7	28.2	28.8
Lying	56.4	59.2	31.9	34.6
Ruminating standing	47.1	42.1	56.6	58.9
Ruminating lying	48.1	40.7	33.4	32.2

There was no difference between the within-pair and between-pair coefficients of variation for any of the behaviours when analysed using either method one or method two (P=0.83, P=0.92, respectively).

Fig. 6.2b shows the distribution of the coefficient of variation values for both within-pairs and between-pairs, for method one, and Fig. 6.3b for method two. For Fig. 6.2b and 6.3b the coefficient data points approximately follow a straight line, illustrating that the coefficient values for both within-pairs and between-pairs were very similar.

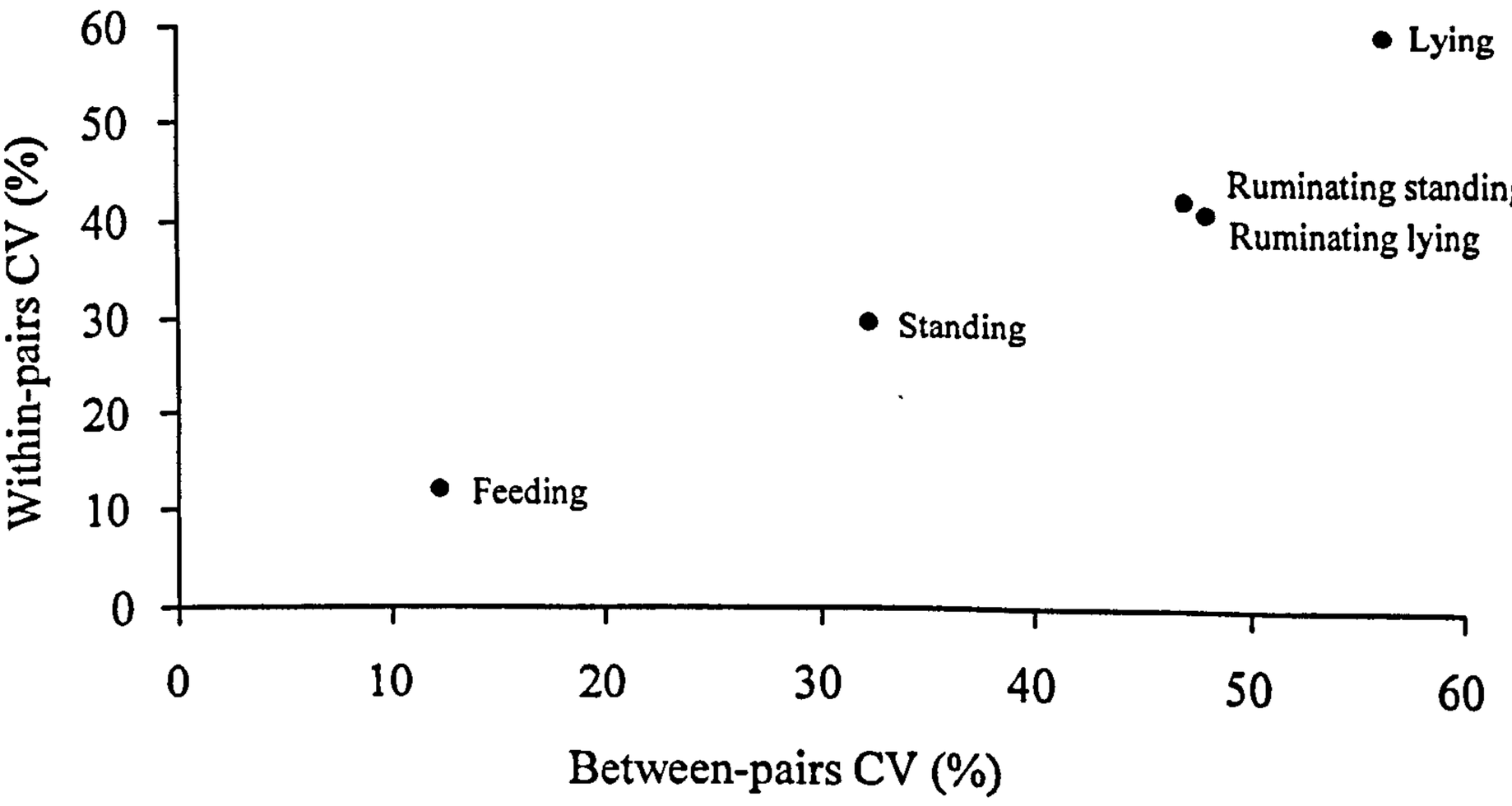


Fig. 6.2b Within-pair coefficient of variation (CV) values plotted against between-pair values (%) for each behaviour in method one

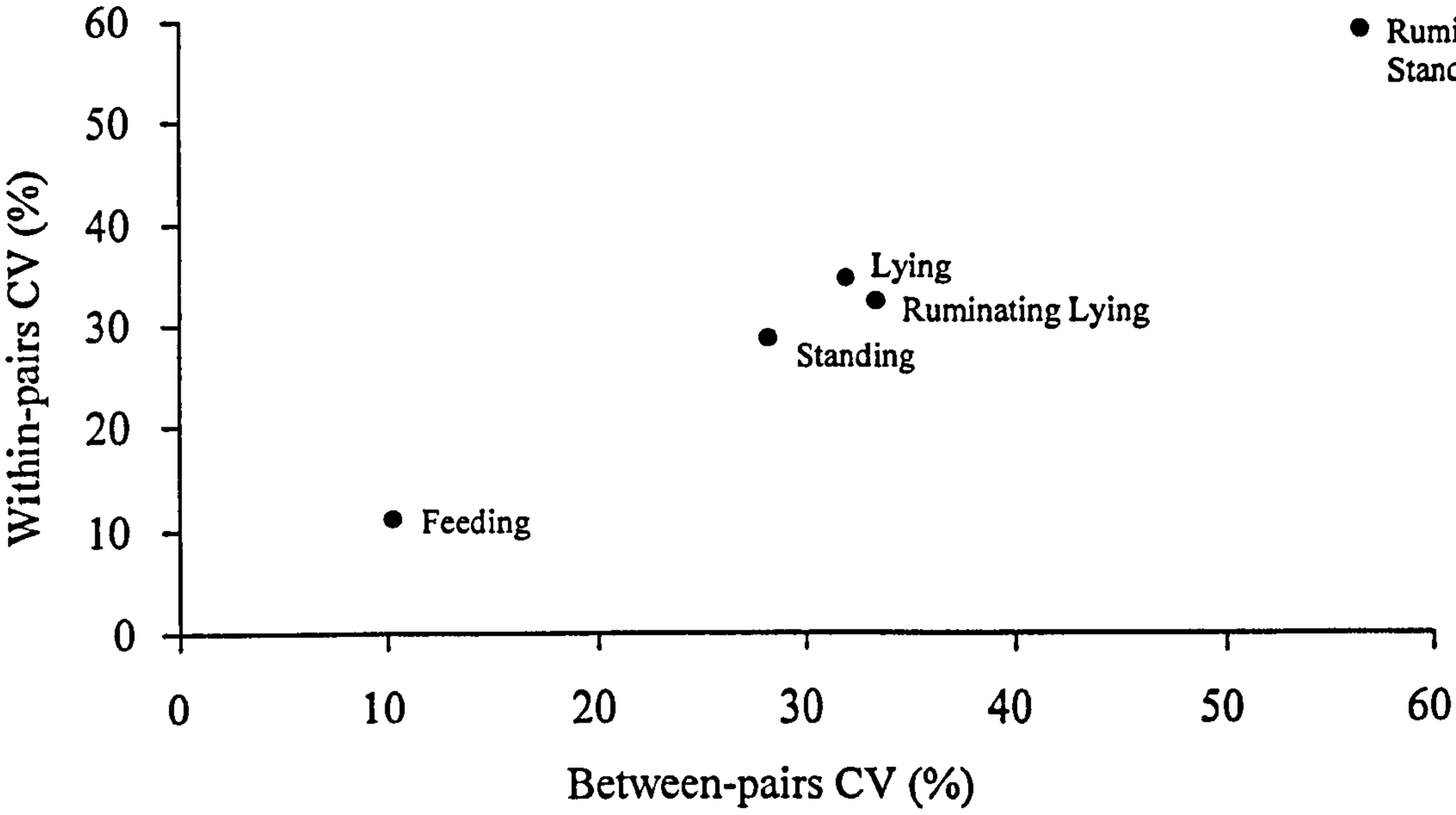


Fig. 6.3b Within-pair coefficient of variation (CV) values plotted against between-pair values (%) for each behaviour in method two

6.5b Discussion

For the cows used in this study, under these conditions, the two methods of analysis revealed that there was no consistent trend for the variation in behaviour between cows within-pairs to be significantly different from that existing between-pairs. This suggests that cows within the same group behaved in a similar fashion to cows not within the same group, which is convincing evidence to suggest that, for the behaviours examined, cows within groups behave independently. The results of this study are in agreement with those obtained in Chapter 5b (The statistical analysis of paired dairy cows I). However, the methods adopted here may have provided a more rigorous examination of interdependence, as pairs of cows had been housed together on up to two occasions previously. For a full discussion of these results see Chapter 5b, section 5.5. In conclusion, these results provide further evidence to support the use of individual cows as replicates in the statistical analysis of their behaviour.

7. General discussion

The primary purpose of this research programme was to examine the effect of a high milk yield on the behaviour and welfare of the dairy cow. Section A of this discussion will highlight the major findings of each experiment and how the results in one experiment lead to the progression of the next in an attempt to address this purpose. This study was also concerned with the legitimacy of using individual dairy cows as replicates in the statistical analysis of their behaviour. The results and implications of these findings will be discussed in section B.

7.1a Introduction: Overview

The study commenced by examining the difference in the behaviour of high and low yielding dairy cows. This experiment was designed to investigate whether, and in what ways, the high yielding dairy cow may have to modify her behaviour in order to cope with the increased nutritional demands of milk production. Cows that were observed not to interact with each other were selected for this study (see section B). There was a tendency for the high yielding cows to increase their bite rate during grazing. The amount of time spent feeding was also observed to increase with milk yield, with the high yielding cows spending significantly more time grazing. The behaviour most significantly reduced by the high yielding cows when grazing was that of lying. This was primarily due to a reduction in the performance of sleeping and lying (not ruminating), which accounted for approximately three quarters of this time. This suggested a trade-off between these behaviours.

A parallel study was set out to measure and compare the appetite for concentrate of high and low yielding dairy cows. This was stimulated by previous research that

suggested the provision of a standard forage ration may not satisfy the appetite of high yielding dairy cows, and to meet their greater energy demands an increased proportion of high energy concentrates may have to be included in their diet (Flachowsky & Lebzien, 1997; O'Connell *et al.*, 2000). If this were true, given the opportunity high yielding cows may demonstrate the need for an additional concentrate allowance in comparison to lower yielding cows. This was explored with the use of a novel operant conditioning technique. Behavioural observations were also conducted on each animal post-testing to reveal whether there were any differences between groups in their motivation to subsequently consume forage. The breaking points achieved, the mean time taken to receive a reward and the mean time taken to receive the first and fourth rewards were similar for the high and low yielding cows, indicating that the high yielding cows did not appear to have a greater appetite for concentrate. It is likely that there came a point where the cows felt that they had spent long enough working for the concentrate, which may have been dictated by the frustration experienced by obtaining further rewards after they had reached a certain level. In addition, the extra time spent having to work for food would mean that there would be less time available to perform other desirable activities, such as rest. However, the time spent forage feeding post-testing and milk yield were positively related, with the high yielding cows spending nearly twice as long feeding compared to the low yielding cows, suggesting a greater appetite.

In the light of the findings of the first study, a third experiment was designed to investigate whether the reduction in lying time being experienced by the high yielding cows when grazing was compromising their welfare. Behavioural observations during deprivation demonstrated behavioural signs of fatigue, frustration and stress, which

were exaggerated with the progression of pregnancy. The negative physical and psychological effects of forced standing, and the motivation to lie post-deprivation were shown to be cumulative, with four hours of lying deprivation resulting in an immediate post-deprivation rebound. Four hours of lying deprivation also reduced milk yields by approximately 0.5 kg cow^{-1}. The results also revealed that higher yielding cows experienced more discomfort due to forced standing.

In the previous study the cows were observed feeding during the deprivation period, allowing them to re-schedule their behaviour by resting during those times when they would have been feeding, post-deprivation, which is not a strategy available to the high yielding dairy cow. This result prompted a fourth experiment, which had the same aims as the third. In the fourth experiment the cows were deprived of both lying and feeding simultaneously. This was to ensure that at the end of the deprivation period the cows would probably be motivated to perform both behaviours, which is likely to mimic that experienced by the grazing high yielding dairy cow. Behavioural observations during deprivation demonstrated behavioural signs of fatigue, frustration and stress. The negative physical and psychological effects of forced standing were cumulative. The motivation to feed exceeded that to lie immediately after being deprived of both behaviours and this was greater in those cows deprived for four hours. Both two and four hours of lying and feed deprivation resulted in an immediate feeding rebound post-deprivation. Although no such rebound was observed for lying, this behaviour was not reduced to extend feeding time, as observed when animals have been deprived of feeding only (Metz, 1985) and as would be expected considering the negative relationship previously observed between these behaviours. The cows deprived of four hours may have increased their rate of feed intake and/or

reduced their preferred feeding time in order to maintain this fixed lying time. Two to four hours of lying and feed deprivation also tended to reduce milk yields by approximately 0.6 kg cow^{-1}. The results also revealed that higher yielding cows and cows closer to parturition experience more discomfort due to forced standing.

7.2a The effect of a high milk yield on behaviour

The most notable impact of a high milk yield was on feeding behaviour. This was expected, as those animals with higher productive outputs have to satisfy their increased energy requirements in some way (Bao *et al.*, 1992). At pasture the high yielding cows primarily achieved this by extending their grazing period. Increased grazing times to increase herbage consumption in high yielding cows have also been observed in other studies (Hancock, 1953; Brumby, 1959; Stobbs, 1970; Lathrop *et al.*, 1988; Phillips & Denne, 1988; Bao *et al.*, 1992; Gibb *et al.*, 1999; Phillips & Rind, 2002). Extended grazing times have also been observed in sheep with increased nutritional demands (Penning *et al.*, 1995).

The high yielding cows also tended to increase their rate of feed intake by increasing bite rate. Increasing the rate of feed intake by eating faster and/or increasing bite size has also been observed in other studies with dairy cows (Manning, 1972; Greenwood & Demment, 1988; Dogherty *et al.*, 1987 & 1989; Patterson *et al.*, 1998; Gibb *et al.*, 1999; O'Connell *et al.*, 2000), sheep (Penning *et al.*, 1995; Iason *et al.*, 1999) and pigs (Day *et al.*, 1995) after a period of fasting. It would appear that both increasing grazing time and bite rate are employed to increase grass intake, as these factors have been shown to be positively related (Hancock, 1953; Spedding, 1966; Gibb *et al.*, 1997).

Increasing biting rate during grazing is a useful strategy, but Phillips and Leaver (1985a) suggested that there is an upper limit to this adaptation of 60-70 bites min^{-1}. As the high yielding cows in the first experiment were approaching this limit (mean of 57.1 bites min^{-1}) it seems there may have been little scope for them to increase this behaviour further. It is expected that this approach can only be utilised to a certain degree and may leave high yielding cows little other option except to extend their grazing period in order to increase herbage intake.

Extending the grazing period will enable a cow to increase her forage intake but at the expense of spending time performing one or more other activities. The behaviour most significantly reduced whilst the cows were grazing was that of lying. This behaviour was shown to be negatively correlated with feeding, indicating a trade-off between these activities whereby one is pursued at the expense of the other. A trade-off between feeding and lying has been observed in other studies with cattle (Atkeson *et al.*, 1942; Castle & Halley, 1953; Veris *et al.*, 1980; Fuerst-Waltl *et al.*, 1999; Fregonesi & Leaver, 2001), sheep (Cockram *et al.*, 1999), pigs (Day *et al.*, 1995) and other species. For example, Dunbar and Dunbar (1988) found that lactating female Gelada baboons (*Theropithecus gelada*) spent more time foraging and less time resting than those baboons with a smaller demand for food. Similarly, Muller-Scharze *et al.* (1982) established that white-tailed deer fawns increase their grazing time at the expense of performing other activities when they experimentally reduced their milk supply. This feeding-lying association in dairy cows may not be so surprising considering the time devoted to these activities occupies a considerable proportion of their day, leaving little else to give up to continue feeding.

There was also a significant reduction in the time spent standing for the high yielding group whilst at pasture. It would seem that this small difference was also linked to the extended grazing period, as no other behaviour was significantly reduced during this time. This was further supported in the lying deprivation experiment by a negative relationship between standing and lying post-deprivation. This suggests that the cows may also have to reduce the time spent standing to some extent to increase their lying time.

7.3a The effect of a high milk yield on welfare

An increased biting rate has been associated with a greater appetite, as studies that have manipulated the intake levels of dairy cows, or subjected them to a period of fasting, have observed (Manning, 1972; Greenwood & Demment, 1988; Dogherty *et al.,* 1987 & 1989; Patterson *et al.,* 1998). Similarly, an extended grazing period has also been shown to be indicative of an increased appetite, as it increases following periods of fasting (Greenwood & Demment, 1988; Patterson *et al.,* 1998; Chillibroste *et al.,* 1997), and reduced intake rates (Manning, 1972). An increased appetite is likely to result from a greater drive to feed. This is supported by Hancock (1953), Baile and McLaughlin (1987) and Phillips (1993) who suggested that the act of grazing itself is used to overcome the sensation of hunger, as it is hunger that motivates the cow to feed. The results of this study therefore propose that that the high yielding cows were experiencing some degree of increased hunger, but this was not determined. However, it may be possible to infer this, in part, from the results of other studies. Patterson *et al.* (1998) recorded that the mean biting rate of lactating dairy cows increased from 44 to 56 bites min^{-1} when cows were previously fasted

from one to 13 hours, respectively. The high yielding cows in experiment one had a mean biting rate of 57 bites min^{-1}, which is close to that of Patterson's *et al.* (1998) cows when fasted for 13 hours, thus possibly suggestive of the relative degree of hunger being experienced by these cows.

As discussed previously, the employment of an extended grazing period compromises the amount of time available to lie. The initial rebound in lying time after four hours of forced standing indicates that the need for lying builds up significantly after only a few hours of lying deprivation, which is also supported by other studies (Metz & Wierenga, 1984; Metz, 1985; Munksgaard & Simonsen, 1996). This was demonstrated further as the motivation to lie in the first hour after milking post-deprivation increased proportionately with increasing time spent deprived of lying. When the cows were deprived of both lying and feeding for four hours, they may have increased their rate of feed intake and/or reduced their feeding time to maintain a fixed lying time. Whichever strategy was adopted they both highlight the importance of lying after only four hours of forced standing. Lying behaviour was not reduced to extend feeding time when the cows were deprived of both. As this has been observed when animals have been deprived of feeding only, and it would be expected to occur considering the negative relationship between these behaviours, this further highlights the importance of maintaining a fixed time for this activity. Lying allows the animal to rest and sleep, which are important for brain health and the recovery of body systems (Fraser & Broom, 1997). The opportunity to satisfy the motivation to lie down is therefore important to ensure the well-being of dairy cows (Metz, 1985; Wierenga & Hopster, 1990; Munksgaard *et al.*, 1999). Its prevention, even for a short

period, can be considered stressful and aversive, poses a risk to cow comfort and should therefore be avoided.

Although a reduction in lying time is detrimental to welfare it is difficult to determine at what point this occurs. A useful reference would be to have some idea of the minimum total lying time required by dairy cows per day. This is difficult to determine as lying times appear to be very flexible and depend on a number of internal and external factors (Wierenga & Hopster, 1990). However, Jensen *et al.* (2002) attempted to determine this, and recorded that cows would work in an operant conditioning test to lie for c. 13 h d^{-1}. The control cows in experiments three and four had mean lying times of c. 14.8 and 14.5 h d^{-1}, respectively. Although greater than 13 h, these cows were forage fed and had relatively low milk yields (24.8 and 21.8 kg d^{-1}, respectively). Both factors will reduce the amount of time spent feeding and therefore increase the amount of time available for lying. In contrast, both the high and low yielding cows in the first experiment were only observed lying for 10.3 and 11.4 h d^{-1}, which are well below the calculated requirement. The high and low yielding groups in this study had mean milk yields of 37.9 and 29.4 kg d^{-1}, respectively. This suggest that a milk yield of c. 30 kg d^{-1} may already exceed the upper level whereby minimum total lying times per day can be achieved under semi-intensive conditions.

During forced standing (Chapters 5a & 6a) the cows demonstrated behavioural signs of fatigue, frustration and stress. These negative physical and psychological effects were cumulative and therefore suggests that the cows were unable to adapt to the situation within the four hour period. The results obtained here are also applicable to situations whereby cows are left standing for long periods, for example, while

awaiting veterinary attention or artificial insemination, when confined to concrete yards during periods of wet weather, or due to the over-occupation of cubicle housing. It was also observed that higher yielding cows and those cows later into their pregnancy experience more discomfort from forced standing. Special attention should be given to these animals in such situations.

It was expected that the reduction in milk yield, observed in experiment three, was symptomatic of standing for a long period in a confined space, rather than being deprived of lying *per se*. Therefore, this effect would be of particular consequence to cows undergoing the conditions named above. Four hours of forced standing reduced milk yields by c. 0.5 kg cow^{-1}, which could have considerable economic consequences. Consider, for example, cows that are awaiting artificial insemination. It is usual practice for such cows to be selected during morning milking and left to stand in pens until the inseminator arrives. Based on a herd size of 400 cows, and a 50% success rate for a confirmed pregnancy using artificial insemination, the annual loss of milk could amount to c. 400 kg.

A reduction in resting time can also have negative effects on other parameters. Munksgaard *et al.* (1999) and Fisher *et al.* (2002) found that repeated lying deprivation increased the cortisol response to an ACTH challenge test. Increased levels of stress, as a direct result of forced standing, can be responsible for lowering immune system function and hence decrease resistance to disease (Fisher *et al.*, 1999). Increased stress can also affect the production of the growth hormone somatotrophin. The prevention of lying can also be responsible for predisposing cows to other health problems such as sole haemorrhages and lameness (Singh *et al.*,

1993a; Leonard *et al.*, 1994; Faull *et al.*, 1996). These factors not only impact on the welfare of the animal, but also on the economic viability of the farmer (Galindo *et al.*, 2000).

7.4a Possible solutions and practical suggestions

A sensible approach would be to identify possible ways in which to reduce the extended grazing period and thus increase the amount of time available for resting. This could be achieved by enabling the cows to increase their rate of dry matter intake. One option would be to offer supplementary rations, which have been shown to reduce grazing activity (Sarker & Holmes, 1974; Leaver, 1985; Phillips, 1993; O'Connell *et al.*, 2000) and increase resting time (Phillips & Leaver, 1986; Munksgaard *et al.*, 2001). Supplementation can be achieved by offering, for example, additional concentrates (O'Connell *et al.*, 2000; Munksgaard *et al.*, 2001), hay or forage (Phillips & Leaver, 1986). O'Connell *et al.* (2000) states that a concentrate supplement is necessary to meet the energy requirements of grazing dairy cows yielding in excess of 25 kg d^{-1}. However, an additional concentrate allowance can present further problems by unbalancing the roughage-to-concentrate ratio and thus predispose the dairy cow to various other health troubles (Kamphues, 1998), for example, acidosis and laminitis. Further, Flachowsky and Lebzien (1997) stated that even when high amounts of concentrate are fed it is difficult to cater for the energy requirements of cows producing in excess of 40 kg milk d^{-1}.

For extensively-managed cows, an increase in sward surface height and density would enable the cow to achieve a greater bite depth therefore increase bite mass and thus dry matter intake rate (Laca *et al.*, 1992; McGilloway *et al.*, 1997; O'Connell *et al.*,

2000). Gibb *et al.* (1999) observed a significant decrease in grazing times with increases in sward heights by as little as 4 cm (*i.e.* from 5 to 9 cm). Intake rates remained the same as those when cows were grazed on a shorter sward but were achieved in a shorter period.

Zero-grazing could also be considered whereby cows are fed a balanced energy ration to complement their yield. This may support the energy requirements of high yielding cows, but at the expense of compromising their welfare in other areas. For example, cows may develop abnormal behaviours such as feed tossing and tongue rolling, which may be redirected grazing activities (Phillips, 2002). Specialist diets can also be consumed in about one half the time of grazed herbage (Phillips, 2000b), providing the cow with additional time that may be difficult to fill in a relatively barren environment. Consequently, excessive grooming, licking and drinking may ensue as a form of stimulation (Kerr & Wood-Gush, 1987; Stimulus Response, 1995). There are also problems associated with the restriction of movement and being in close confinement with other cows.

The most obvious approach, which may also bring about the greatest improvements to animal welfare, would be to impose an upper limit to milk yield. The FAWC, in 1997, suggested that 'production may have already passed the point where good cow welfare can be maintained.' In a recent Delphi questionnaire (2002), conducted by the Edinburgh Team at SAC, experts were asked to consider and rate the impacts on welfare of specified changes to breeding in dairy cows. They indicated that to impose a maximum daily/lactation yield would have a very high welfare improvement (rating this 9 out of 10, whereby 10 is the maximum rating for welfare improvement). They

also indicated that halting selection for milk yield and reversing the selection for milk yield would bring about moderate to high welfare improvements, respectively (rating them 5 and 7, respectively). If accepted, these would be long-term goals.

In the short-term, to improve the welfare of the high yielder a system involving grazing with additional forage supplementation could be used (Phillips & Leaver, 1985a). This would allow behaviour to be expressed relatively unrestricted and an adequate nutrient intake to be achieved. It is likely, then, that a semi-intensive management system may provide the best compromise between the farmer's requirement to maximise milk yields and the cow's requirement for an open environment.

7.5a Retrospective suggested improvements and future research

A milk yield of 25 kg d^{-1} has been suggested as the threshold whereby cows yielding in excess of this will require concentrate supplementation to meet their nutrient requirements (O'Connell *et al.*, 2000). The mean milk yield for the high yielding group in the second experiment was 26.4 kg d^{-1}, which may not have been great enough for a significant need for concentrate to be expressed. Thus it would have been beneficial to use cows with greater milk yields for the high yielding group.

The actual lying deprivation times of the cows that underwent two hours of deprivation in experiments three and four closely mimicked that experienced by the grazing high yielding cows in the first experiment (c. 80 and 50 versus c. 60 min, respectively), adding value to the validity of the transference of these results to such cows. However, there were potential confounding factors that could make a direct

comparison implausible. For example, the cows were deprived of lying in one bout. As lying is a polyphasic activity, it is more likely that high yielding cows have a number of shorter lying periods in comparison to their lower yielding counterparts. The experiment also only examined the effects of lying deprivation for one day. It is likely that grazing high yielding dairy cows are deprived of lying every day, possibly for the duration of their lactation. This may lead to cumulative effects, which could not be observed in this study. A further dissimilarity was that the deprivation of lying took place within a small area, which restricted movement. This would not be a situation experienced by grazing dairy cows. It is probable that some of the behaviours expressed, such as leg raising and weight shifting, were symptomatic of standing for long periods in a confined space, rather than being deprived of lying *per se*. In addition, other behaviours such as self grooming, rubbing the head against the housing, body care and licking may have been performed as a form of stimulation in response to being in a relatively barren environment (Kerr & Wood-Gush, 1987; Stimulus Response, 1995). One further research problem was that the priority to feed, rather than lie, post-deprivation, may be related to the timing of the deprivation period. The first eight hours after deprivation included a post-milking period, when a cow's motivation to feed is usually greater than that to lie (Phillips & Leaver, 1986), which may have subsequently influenced the results. Although it was necessary to impose such experimental restrictions to overcome possible problems with statistical analysis of the data and to ensure, as humanely as possible, the prevention of lying, more transferable results may have been obtained with grazing dairy cows. The prevention of lying in an extensive situation could have been achieved, if ethically acceptable, by fitting the cows with girth straps that would emit an electrical stimulus when the cows would lie. This has been successfully used to prevent cows from lying

by other researchers (Fisher *et al.*, 2002). Such a device would enable the cows to remain as part of the herd and undergo management practices in the normal way. The stimulus could be switched on or off to deprive the cows of lying at different times of the day and to extend periods of standing throughout the day to reflect the polyphasic nature of lying. The straps could also remain on the cows to simulate daily lying deprivation.

It would have been beneficial to measure the rate of forage intake for the cows undergoing the lying and feeding deprivation experiment to determine whether this was a strategy they adopted to increase feed intake post-deprivation. This could be conducted with the use of a computerised feeding system that monitors the amount of food consumed by each cow over time. Behavioural measures such as bite and chewing rate of food could also be taken.

Further research should also aim to: (1) determine the extent to which the lying deprivation phase continues during the lactation period, and its intensity at certain stages of lactation; and (2) determine minimum resting times required by dairy cows to maintain full health and welfare. This information could then be used to specify optimum milk yield levels that enable cows to cope under both extensive and semi-intensive environments, before their welfare is further compromised. This could be achieved in one way by observing the maximum effort cows are prepared to give to continue lying after a given period of time spent in this behaviour, for example, by using a lever pressing device. During periods of rest, cows could be made to stand and emit a given number of presses in order to continue lying. This could also be conducted at different times of the day and also at different stages of lactation. The

results of this experiment could perhaps be compared with the motivation of high yielding cows to lie after a day's grazing, which could also be determined by using this method. The operant device used in the second study would be suited to this purpose. The cows were easily trained with this device within a short period. An alternative, and maybe simpler method, could be to use an aversion test. For example, cows could only be given the opportunity to lie on a surface that they find less pleasant, such as concrete (Fisher *et al.*, 2003) possibly covered with slurry. As the cows would presumably want to minimise the amount of time spent lying on such a surface, this may provide some indication of minimum total lying times per day. However, it may be difficult to determine an absolute minimum lying time for dairy cows. It may therefore also be beneficial to tackle this question from an alternative angle, and efforts could be placed towards attempting to establish an optimum milk yield. This could be decided in part by seeing at which point grazing (on a standard quality pasture) alone is not sufficient to cater for the nutritional requirements of the lactating cow, whereby supplementary rations would be necessary in order to maintain full health and welfare. This value is estimated to be approximately 25 kg cow^{-1} (O'Connell *et al.*, 2000).

There is always a problem with the construal of behaviours. In particular, in the lying deprivation study, it was difficult to interpret exactly what the rebound result meant in terms of welfare to the animal, *i.e.* how much of a rebound constitutes suffering (Dawkins, 1988). This would highlight the need to conduct a variety of tests, which set out to investigate the same question. Behavioural recordings are only one method available to evaluate welfare. Other techniques need to be considered in conjunction with these to make an accurate and reliable welfare judgement.

7.6a General conclusion

Whilst grazing, there were two strategies that the high yielding cows adopted to increase forage intake. These were to increase the amount of time spent grazing, and increase biting rate. As similar behavioural responses have been observed in fasted cattle, and comparable biting rates have been recorded for cattle fasted for 13 hours, it is reasonable to propose that the high yielding cows used in this experiment were experiencing some degree of hunger. In an attempt to achieve satiety, the high yielding cows primarily extended their grazing period. A trade-off between feeding and lying time has been observed whereby one is pursued at the expense of the other. Since feeding probably induces such a powerful motivational force, the drive to feed may almost always override that to rest, until satisfied. Evidence is offered that demonstrates that the deprivation of lying can be detrimental to welfare, thus a reduction in this behaviour below a certain threshold is likely to result in suffering. This threshold remains largely undetermined, but a current best estimate predicts this to be approximately 13 h d^{-1} (Jensen *et al.*, 2002). The cows used in the first study, which were managed semi-intensively and had a mean milk yield of c. 30 kg d^{-1}, only achieved a lying time of c. 11.4 h d^{-1}. This suggests that a milk yield of this value may already exceed the upper level whereby minimum total lying times per day can be achieved, and good welfare can be maintained. As it has been shown that the amount of time spent lying decreases as milk yield increases (Chapters 3b, 4 & 6a; Fuerst-Waltl *et al.*, 1999; Chaplin & Munksgaard, 2001; Fregonesi & Leaver, 2001), continuing to breed for greater milk yields may be to the further detriment of dairy cow welfare. Consideration should therefore be given towards identifying and imposing an upper limit to milk yield, which may allow for an optimum lying time to be achieved. However, such a suggestion should take into account other factors that

influence lying time (Section 5.1a). Further research to investigate these proposals have been suggested, which would aid the determination of minimum lying times and optimum milk yields, thus going some way to the further improvement of dairy cattle welfare.

7.1b Introduction: Overview

The use of individual dairy cows in the statistical analysis of their behaviour has received much attention and is fervently debated. The basis of this argument is the question of whether dairy cows behave interdependently. For example, Rook and Huckle (1995) believe that the grazing behaviour of dairy cows may be socially facilitated, with the commencement and duration of this period being dependent on a few individuals within the group. If shown, this could have implications for the legitimate use of ANOVA, which requires that replicates are independent of each other (Snedecor & Cochran, 1978). However, the existence of strong evidence to support this contention is absent. The experimenter took into consideration such effects when designing experiments, and conducted three studies to determine whether dairy cows can be considered as behaving autonomously.

The first of these experiments set out to determine whether cows within a group would associate with each other more than would be expected by chance, thus possibly indicating interdependent behaviour between individuals. The positions of 60 cows within a group were recorded on 24 separate occasions when they were either lying down, predominantly feeding or entering the milking parlour. These were times when the cows could chose who they interacted with, therefore those cows that were observed to associate frequently could be regarded as behaviourally interdependent.

For the feeding data, a predictive feeding association model (Webb, 2002) revealed that the cows were mixing at random. In contrast there was a high level of association between individuals on entering the milking parlour. When the data were combined for all measures, 85% of the cows were observed with another cow on at least two occasions. Forty one percent (*i.e.* 21 cows) of these cows were observed with another cow on three occasions, and no cow was observed with another more than three times. This scale of pairings was likely to have occurred at random. Those cows that were not observed to interact with each other were selected to be used in the first experiment of section A.

The methodology used here offered a useful starting point for the study of interdependence. For, before interdependence can be studied, it is necessary to determine whether cows within a group are associated. As partnerships may be superficial and change with time, as is seen with order of entry into the parlour during the course of a lactation, lengthy studies are required to confirm whether observed associations are genuine. It is the impact of the associations that then needs to be examined to determine whether the behaviour of those individuals is affected. This could be achieved by comparing the behaviour of identified associates within a group with other non-associated group members to determine whether the associated cows were more similar in their behavioural patterns. This suggestion was carried forward and formed the basis of the following two studies.

The aim these two studies was to determine whether dairy cows, whilst performing behaviours such as feeding, standing, and lying, can be considered to be acting independently. Behaviours, such as allogrooming, aggression and yawning, can be

considered as interdependent. Conversely, respiration, for example, is almost certainly independent. However, feeding, standing and lying were of most interest because they are not so easily categorised and are the subject of controversy. Cows were housed in pairs, hence there were individuals that could interact with each other (within-pairs) and individuals that were unable to influence each other's behaviour (between-pairs). The experimental design of both deprivation experiments in section A facilitated this approach and thus data from these studies were used for analysis. For the behaviours examined the variation between individuals within a pair was compared with the variation between individuals from different pairs. Both studies utilised two different data sets for analysis. Both studies revealed that there was no consistent trend for the variation in behaviour between cows within-pairs to be significantly different from that existing between-pairs. This suggests that the behaviour of cows within the same pair is similar to those not within the same pair. The results of these enquiries formed the basis for the statistical analyses methods selected for data exploration in the third and fourth experiments in section A.

7.2b Retrospective suggested improvements and future research

The order of entry into the milking parlour may not have been fully suited towards detecting the occurrence of interdependence. The cows entered the parlour in a similar order for each observation, suggesting that they were consistent in their interactions between individuals. However, it is not possible to determine the nature of these interactions. For example, this does not imply that the order was determined by each cow's preference to be in close contact with another individual, or, that there was some order being manipulated by certain cows within the group: Such as is found in a dominance hierarchy. Nevertheless, it could be used to support the occurrence of

independent behaviour. If cows were acting interdependently, they may be expected to enter the parlour with the same group members over time, irrespective of other effects, but would only be the case if sociality had priority over the need to be milked. Further research is required to validate this assumption.

Although convincing evidence is provided for the justification of the use of individual cows in data analysis, some factors should be taken into account before applying the results to alternative data sets. The results obtained may be specific to this study as they may vary with factors such as group size, the length of time the cows were together, the behaviours studied, and the experimental conditions. The cows within this study were housed in pairs. This does not truly reflect the 'normal' on-farm situation. Feral cattle are social animals and typically sub-group into approximately 10-12 individuals (Phillips, 1993). Cows also behave differently under extensive conditions and tend to synchronise their grazing and lying behaviour more strongly (Atkeson *et al.*, 1942). To fully examine the conclusiveness of these results, an examination of interdependence could take place using several larger, non-interacting groups of grazing dairy cows. Measurements could be taken of the time spent in various behaviours for each cow over a given time period, after dominance hierarchies have been established. The amount of variation existing between individuals within groups could be compared with that of individuals not within the same group, to determine whether cows within groups were more similar in their performance of the observed behaviours, which would be indicative of interdependence. An alternative method would be, for example, to have five non-interacting groups of five grazing dairy cows and record the behaviour of all cows in all groups, after dominance establishment. Then, re-allocate each member of each

group to form five new groups of individuals that have not been together previously, and measure their behaviour as before. This procedure could be repeated up to five times. This data could be used to determine whether the behaviour of each cow altered significantly.

7.3b Implications and general conclusion

The results of the first experiment could not be used to argue in favour of genuine associations. There was therefore no strong evidence to suggest behavioural interdependence, as an association would be an expected prerequisite for this to occur. The second and third studies demonstrated that the behaviour of cows within the same group was similar to that of those not within the same group, which is convincing evidence to suggest that, for those behaviours examined, cows within groups behave independently. In conclusion, the results of these studies cannot be interpreted to support interdependence between cows of those behaviours examined, and, in particular, for feeding, standing and lying. This therefore would support the use of individual cows as replicates in the statistical analysis of the behaviours examined. This renders current cautionary measures in data manipulation, which could lead to reduced sensitivity and misleading results, unnecessary. In addition, this may remove the need to cater for such effects in experimental design, thus removing those constraints that allow the experimenter to imitate more realistic situations. The limitations to the application of these results have been discussed and further research to confirm these suggestions presented.

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List of published papers from the work of this thesis

1. Cooper, M.D. (2003). The impact of a high milk yield on the welfare of dairy cows. Animal Focus, Moulton College, p5.

2. Cooper, M.D., Phillips, C.J.C. and Arney, D.R. (2003). The motivation of high and low yielding dairy cows for supplementary concentrate feed. In: Ferrante, V., Canali, E., Carenzi, C., Mattiello, S., Minero, M., Palestrini, C., Tosi, M.V., and Verga, M. (eds). *Proceedings of the International Society for Applied Ethology*, Italy, June 24-28th, p146

3. Cooper, M.D., Arney, D. and Phillips, C.J.C. (2002). Differences in the behaviour of high and low yielding dairy cows. *Proceedings of the British Society of Animal Science*, York, UK, April, p220

Appendix 1.

Distance matrix for interaction data showing the number of times each cow was observed with every other cow over 24 recorded occasions.

Cow No.	3	4	5	11	14	19	21	36	37	42	52	60	65	72
3	0	0	1	2	1	0	0	0	0	2	1	0	1	0
4	0	*	0	0	0	0	2	0	0	0	0	0	1	0
5	1	0	*	0	0	0	0	0	2	0	1	0	0	0
11	2	0	0	*	2	0	1	0	1	1	0	0	0	0
14	1	0	0	2	*	0	0	0	0	0	0	1	0	0
19	0	0	0	0	0	*	0	0	1	0	1	0	0	1
21	0	2	0	1	0	0	*	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	*	0	0	0	0	0	0
37	0	0	2	1	0	1	0	0	*	0	0	1	0	0
42	2	0	0	1	0	0	0	0	0	*	2	0	0	0
52	1	0	1	0	0	1	0	0	0	2	*	1	0	0
60	0	0	0	0	1	0	0	0	1	0	1	*	0	0
65	1	1	0	0	0	0	0	0	0	0	0	0	*	0
72	0	0	0	0	0	1	0	0	0	0	0	0	0	*
74	0	0	0	0	0	1	1	1	0	1	1	0	0	0
77	0	0	0	0	0	0	0	0	0	0	1	0	0	0
81	0	0	0	0	0	1	0	1	0	0	0	0	0	1
82	1	0	0	1	0	1	0	0	1	0	0	0	0	0
89	1	1	1	1	0	1	0	0	0	0	1	0	0	1
95	0	0	0	1	0	1	0	0	0	0	0	0	0	2
101	1	0	0	0	1	0	0	0	1	0	0	0	0	0
106	0	0	0	1	0	1	0	0	0	1	0	1	1	0
112	0	0	0	0	0	0	0	0	1	0	1	1	0	0
122	0	0	0	0	1	0	0	0	1	1	0	0	0	0
123	1	0	0	0	0	0	0	0	0	0	0	1	0	0
129	2	0	0	0	0	0	0	1	1	0	0	0	1	0
138	0	0	0	0	0	0	0	0	1	0	0	0	0	1
143	1	1	0	0	0	2	0	0	0	0	0	0	0	0
152	0	0	0	0	0	1	0	0	0	0	1	0	0	0
180	0	0	0	0	0	0	0	0	0	1	0	0	1	0
181	0	0	0	0	1	0	1	0	0	0	0	0	0	0
185	0	0	0	0	1	1	0	0	0	0	0	0	0	0
210	0	0	0	3	0	0	0	0	0	0	0	0	0	0
230	0	2	1	0	1	0	0	1	1	0	0	0	0	0
236	1	1	0	0	0	0	0	1	0	0	0	2	0	0
300	1	0	0	0	0	0	0	0	0	0	0	0	0	0
302	1	1	0	0	1	0	0	0	0	0	0	0	0	0
303	0	1	0	0	0	0	0	0	0	0	0	1	0	0
509	0	0	0	0	0	0	0	1	0	0	1	1	0	0
527	0	0	1	1	0	1	0	0	0	0	0	0	0	0
537	0	0	1	0	0	0	0	1	0	0	0	0	0	1
540	0	1	0	1	0	0	0	0	0	0	1	1	0	0
543	0	0	0	0	1	1	0	0	0	0	1	0	0	0
544	1	0	0	0	1	1	0	2	0	0	2	0	0	2
545	0	0	1	1	0	0	0	0	0	0	0	0	0	0

549	1	0	0	0	2	1	0	1	0	0	1	0	1	0
551	0	0	0	0	0	1	0	0	0	1	0	0	0	0
552	0	0	0	0	0	0	1	1	1	0	1	0	0	0
553	0	0	0	0	1	0	0	1	0	0	0	2	0	0
557	0	0	0	0	0	0	0	1	0	0	0	0	0	0
559	0	0	0	0	0	0	0	0	0	0	0	0	0	2
561	0	0	0	0	0	0	1	0	1	0	0	1	0	0
563	0	0	0	0	0	0	0	0	1	0	0	0	0	0
567	0	0	0	0	0	1	0	1	0	0	0	0	0	0
572	0	0	0	0	0	0	0	0	1	0	0	0	0	0
577	0	1	0	0	0	0	0	1	0	1	0	0	0	0
579	0	0	1	0	0	0	1	0	0	0	0	1	0	1
588	0	1	1	0	0	0	0	0	0	1	1	1	0	0
697	0	0	0	1	0	1	0	0	0	0	0	0	0	1
699	0	1	0	0	0	1	1	0	1	0	0	0	0	0

74	**77**	**81**	**82**	**89**	**95**	**101**	**106**	**112**	**122**	**123**	**129**	**138**	**143**	**152**
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0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
1	0	1	1	1	1	0	1	0	0	0	0	0	2	1
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
0	0	0	1	0	0	1	0	1	1	0	1	1	0	0
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1	1	0	0	1	0	0	0	1	0	0	0	0	0	1
0	0	0	0	0	0	0	1	1	0	1	0	0	0	0
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0	0	1	0	1	2	0	0	0	0	0	0	1	0	0
*	0	0	0	0	1	0	0	1	1	0	0	0	0	0
0	*	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	*	0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	*	0	0	1	1	0	0	0	0	0	0	0
0	0	0	0	*	0	0	0	0	0	1	0	1	0	0
1	0	1	0	0	*	0	0	0	0	1	0	0	0	0
0	0	0	1	0	0	*	1	1	0	2	0	0	2	2
0	0	0	1	0	0	1	*	0	0	0	1	0	1	0
1	0	0	0	0	0	1	0	*	0	0	1	0	0	0
1	0	0	0	0	0	0	0	0	*	0	1	0	0	1
0	0	0	0	1	1	2	0	0	0	*	1	0	1	0
0	0	0	0	0	0	0	1	1	1	1	*	0	0	0
0	1	0	0	1	0	0	0	0	0	0	0	*	0	0
0	0	0	0	0	0	2	1	0	0	1	0	0	*	0
0	0	0	0	0	0	2	0	0	1	0	0	0	0	*
0	0	0	0	1	0	2	0	0	0	0	0	0	0	0
0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	2	0	0	1	0	1	1	0
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0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
1	0	1	0	0	1	1	2	1	1	1	0	0	1	1
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0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
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0	0	0	1	0	0	0	0	1	0	0	0	0	1	0
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1	0	0	0	0	1	0	1	0	1	1	1	0	1	0

180	181	185	210	230	236	300	302	303	509	527	537	540	543	544
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0	0	0	0	2	1	0	1	1	0	0	0	1	0	0
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0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	0	0	0	1	0	1	0	0	2
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0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
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0	0	0	0	0	1	1	0	0	0	1	0	0	1	0
2	1	0	0	0	0	1	0	0	0	0	0	0	0	1
0	0	0	2	0	1	2	0	1	0	0	1	0	0	0
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0	0	0	0	1	0	1	0	1	0	0	0	0	0	1
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0	0	0	1	1	0	1	0	0	0	0	0	0	1	0
0	1	0	0	0	0	1	0	0	1	0	0	0	0	0
*	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	*	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	*	0	0	0	0	1	0	0	0	1	0	0	0
0	0	0	*	0	0	1	0	0	0	0	1	1	0	0
0	0	0	0	*	0	1	1	0	0	0	1	0	0	0
0	0	0	0	0	*	0	0	0	0	0	0	0	0	1
0	0	0	1	1	0	*	0	0	0	0	0	0	0	0
0	0	1	0	1	0	0	*	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	*	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	*	0	0	1	0	0
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0	0	1	1	1	0	0	0	0	0	0	*	0	0	0
0	0	0	1	0	0	0	0	0	1	0	0	*	0	1
0	0	0	0	0	0	0	0	1	0	0	0	0	*	0
0	0	0	0	0	1	0	0	0	0	0	0	1	0	*
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0	0	0	0	0	1	1	0	1	1	1	0	0	2	2
0	0	1	0	0	1	0	0	0	0	0	1	0	0	0
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0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
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2	1	0	1	0	0	0	0	1	1	0	0	0	0	0
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0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
0	1	0	0	1	0	0	0	0	0	0	1	0	0	0
0	0	1	0	0	0	0	0	0	1	0	0	0	0	0

545	549	551	552	553	557	559	561	563	567	572	577	579	588	697	699
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1
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0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1
0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1
0	1	0	1	1	1	0	0	0	1	0	1	0	0	0	0
0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	1
0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0
0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
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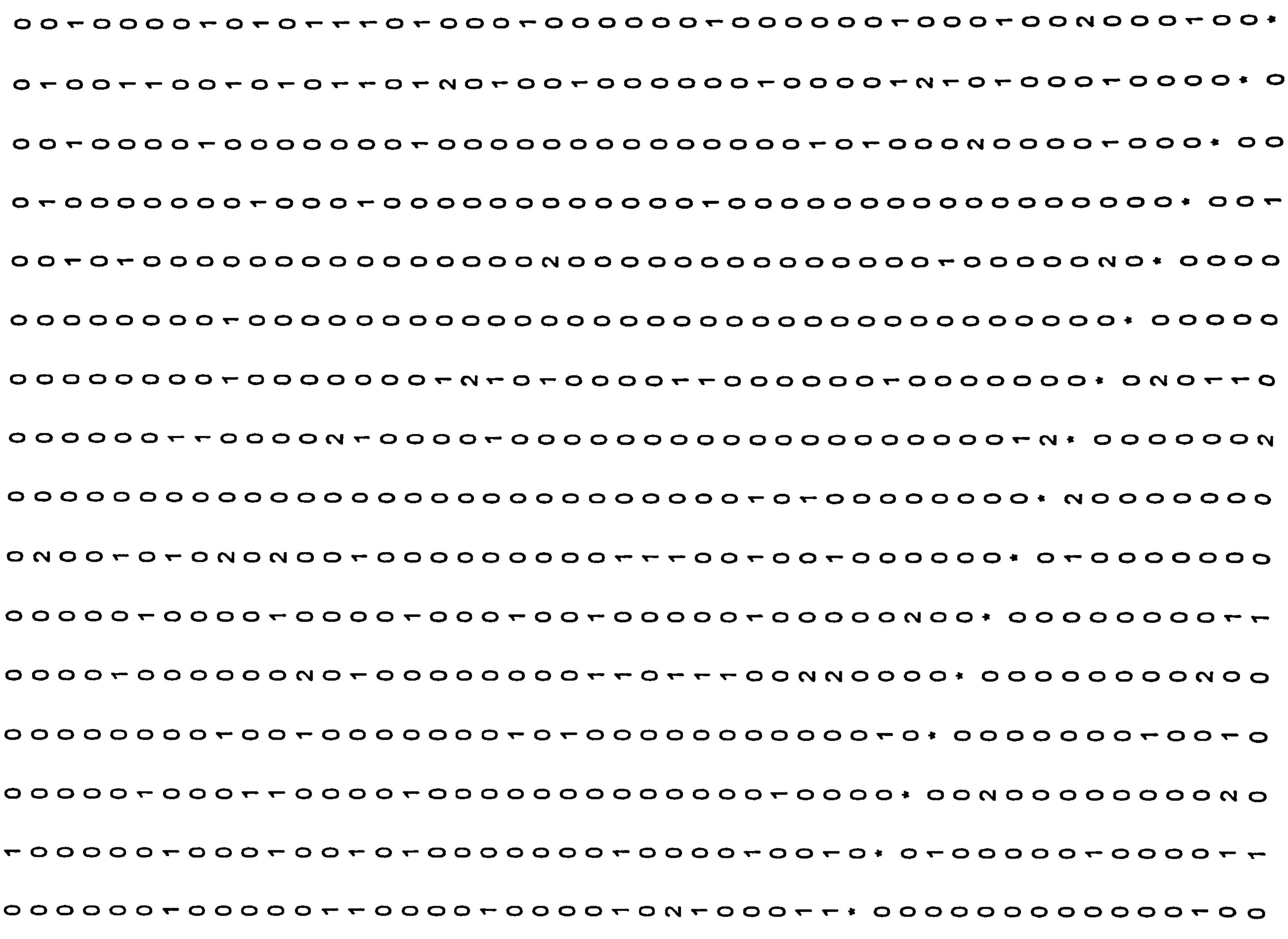

Appendix 2.

Dendrogram data output for interaction data. Output shows the cows clustered within a group according to their level of similarity (%) of interactions with other individuals within the group.

Amalgamation Steps: Euclidean Distance, Complete Linkage

Step	Number of clusters	Similarity level	Distance level	Cows joined		Cluster number	Number of obs. in new cluster
1	60	94.34	1.414	60	61	60	2
2	59	93.07	1.732	16	55	16	2
3	58	89.41	2.646	16	37	16	3
4	57	88.68	2.828	13	16	13	4
5	56	87.99	3.000	17	40	17	2
6	55	87.34	3.162	13	32	13	5
7	54	86.72	3.317	38	58	38	2
8	53	86.72	3.317	27	57	27	2
9	52	86.13	3.464	31	52	31	2
10	51	86.13	3.464	38	39	38	3
11	50	85.57	3.606	13	50	13	6
12	49	85.57	3.606	27	42	27	3
13	48	85.57	3.606	18	29	18	2
14	47	85.02	3.742	15	17	15	3
15	46	84.50	3.873	41	53	41	2
16	45	84.50	3.873	18	23	18	3
17	44	84.50	3.873	1	22	1	2
18	43	83.99	4.000	38	43	38	4
19	42	83.99	4.000	7	34	7	2
20	41	83.99	4.000	13	31	13	8
21	40	83.49	4.123	30	56	30	2
22	39	83.49	4.123	18	28	18	4
23	38	83.02	4.243	8	24	8	2
24	37	82.55	4.359	18	47	18	5
25	36	82.55	4.359	27	45	27	4
26	35	82.55	4.359	26	36	26	2
27	34	82.55	4.359	19	20	19	2
28	33	82.55	4.359	13	15	13	11
29	32	82.55	4.359	3	10	3	2
30	31	82.10	4.472	25	51	25	2
31	30	82.10	4.472	35	38	35	5
32	29	81.22	4.690	14	41	14	3
33	28	81.22	4.690	7	13	7	13
34	27	80.80	4.796	6	59	6	2
35	26	80.80	4.796	8	27	8	6
36	25	80.39	4.899	7	35	7	18
37	24	80.39	4.899	1	18	1	7
38	23	79.98	5.000	3	19	3	4
39	22	79.98	5.000	5	9	5	2
40	21	79.20	5.196	44	46	44	2
41	20	79.20	5.196	7	30	7	20
42	19	79.20	5.196	14	26	14	5
43	18	79.20	5.196	8	11	8	7
44	17	78.07	5.477	3	54	3	5
45	16	78.07	5.477	8	49	8	8
46	15	77.71	5.568	1	7	1	27
47	14	77.71	5.568	2	3	2	6
48	13	77.35	5.657	14	44	14	7
49	12	77.35	5.657	5	33	5	3
50	11	76.66	5.831	6	21	6	3
51	10	76.66	5.831	1	2	1	33

Continued...

Step	Number of clusters	Similarity level	Distance level	Cows joined		Cluster number	Number of obs. in new cluster
52	9	75.65	6.083	1	5	1	36
53	8	75.32	6.164	8	25	8	10
54	7	75.00	6.245	1	14	1	43
55	6	73.15	6.708	1	4	1	44
56	5	72.26	6.928	6	60	6	5
57	4	71.41	7.141	1	8	1	54
58	3	69.25	7.681	1	6	1	59
59	2	49.52	12.610	1	48	1	60
60	1	0.00	24.980	1	12	1	61

Final Partition

Number of clusters: 1

	Number of observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster1	61	1351.115	3.935	21.584